UNIVERSIDADE FEDERAL DE LAVRAS

RAFAEL AGOSTINHO FERREIRA

FROM SEEDS TO PLANTS: THE USE OF PRIMING TO IMPROVE SALT TOLERANCE IN SORGHUM

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DE SEMENTES A PLANTAS: O USO DO *PRIMING* PARA AUMENTAR A TOLERÂNCIA DO SORGO À SALINIDADE

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Agronomia/Fisiologia Vegetal, área de concentração em Fisiologia Vegetal, para obtenção do título de Doutor.

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No te des por vencido, ni aun vencido,
no te sientas esclavo, ni aun esclavo;
trémulo de pavor, piénsate bravo,
y arremete feroz, ya mal herido.
Ten el tesón del clavo enmohecido
que ya viejo y ruin, vuelve a ser clavo;
no la cobarde estupidez del pavo
que amaina su plumaje al primer ruido.
Procede como Dios que nunca llora;
o como Lucifer, que nunca reza;
o como el robledal, cuya grandeza
necesita del agua y no la implora...
Que muerda y vocifere vengadora,
ya rodando en el polvo, tu cabeza!
¡PIU AVANTI! – Almafuerte

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RESUMO

Diversas condições ambientais podem restringir a germinação e o estabelecimento de plantas, sendo a salinidade elevada uma das mais críticas. Essa condição decorre da alta concentração de íons no solo, que provoca alterações metabólicas essenciais, afetando tanto a germinação quanto o desenvolvimento das plantas. A salinidade gera dois tipos de estresse: (i) osmótico, causado pela redução do potencial osmótico da solução do solo, e (ii) iônico, devido à dissociação de íons, que danifica membranas e outras estruturas celulares. Em regiões semiáridas e áridas, a necessidade de cultivar espécies tolerantes à salinidade é urgente, e o sorgo (Sorghum bicolor (L.) Moench), embora moderadamente tolerante, possui estágios críticos, como a germinação e o estabelecimento de plântulas, que são altamente sensíveis aos efeitos salinos. Uma alternativa promissora para mitigar esses efeitos é a técnica de priming, que melhora a germinação e o desenvolvimento das plantas. Este trabalho investigou a hipótese de que o priming com moléculas antioxidantes e fitoreguladores oferece melhores condições para a germinação e o crescimento do sorgo sob estresse salino. Para compreender os mecanismos de tolerância, foram realizados experimentos em laboratório e em casa de vegetação. No primeiro capítulo, avaliou-se a resposta de sementes de quatro variedades de sorgo submetidas ao priming com ácido ascórbico, ácido abscísico e hidropriming, expostas a diferentes concentrações de NaCl e estresse osmótico induzido por PEG-6000. Os testes analisaram parâmetros como porcentagem de germinação, índice de velocidade de germinação e tempo médio para germinação de 50% das sementes. No segundo capítulo, realizaram-se análises bioquímicas em duas cultivares contrastantes (BRS-332 e DKB 540), avaliando o metabolismo de carboidratos e aminoácidos, níveis de peróxido de hidrogênio e malondialdeído, além da atividade de enzimas antioxidantes. Já o terceiro capítulo abordou experimentos em casa de vegetação, nos quais as plantas oriundas de sementes tratadas com priming foram acompanhadas por 65 dias sob estresse salino. Parâmetros como fluorescência da clorofila *a*, trocas gasosas e marcadores relacionados à tolerância à salinidade identificados nas sementes foram analisados em diferentes estágios fenológicos. Os resultados evidenciaram que as cultivares possuem mecanismos distintos de tolerância ao estresse osmótico e salino. O priming promoveu efeitos positivos, especialmente pelo acúmulo de prolina e açúcares, que facilitaram o ajustamento osmótico e mitigaram o estresse oxidativo em sementes, plântulas e plantas adultas. Esses efeitos podem ser atribuídos à "memória do priming", um fenômeno que permite a recuperação de alterações metabólicas induzidas pelo tratamento inicial, resultando em melhor manutenção do metabolismo fotossintético e redução dos impactos do estresse salino ao longo dos diferentes estágios fenológicos. Dessa forma, o priming se apresenta como uma ferramenta eficaz para melhorar a tolerância do sorgo ao estresse salino, contribuindo para o desenvolvimento de estratégias sustentáveis que permitem o cultivo em áreas salinizadas, ampliando o potencial produtivo e a resiliência agrícola em regiões adversas.

Palavras-chave: estresse oxidativo; mudanças climáticas; *hormopriming;* estresse abiótico; prolina.

ABSTRACT

Various environmental conditions can restrict the germination and establishment of plants, with high salinity being one of the most critical. This condition arises from the high concentration of ions in the soil, leading to essential metabolic alterations that affect both germination and plant development. Salinity induces two types of stress: (i) osmotic stress, caused by the reduction of the osmotic potential in the soil solution, and (ii) ionic stress, resulting from ion dissociation, which damages membranes and other cellular structures. In semi-arid and arid regions, there is an urgent need to cultivate species with some level of salt tolerance. Sorghum (*Sorghum bicolor* (L.) Moench), although moderately tolerant, exhibits critical stages such as germination and seedling establishment, which are highly sensitive to salinity effects. A promising approach to mitigating these effects is the priming technique, which enhances germination and plant development. This study investigated the hypothesis that priming with antioxidant molecules and growth regulators provides better conditions for sorghum germination and growth under salt stress. To understand the tolerance mechanisms, experiments were conducted both in laboratories and greenhouse conditions. In the first chapter, the response of seeds from four sorghum varieties subjected to priming with ascorbic acid, abscisic acid, and hydropriming was evaluated under different NaCl concentrations and osmotic stress induced by PEG-6000. Tests analyzed parameters such as germination percentage, germination speed index, and the mean time for 50% seed germination. In the second chapter, biochemical analyses were performed on two contrasting cultivars (BRS-332 and DKB 540), assessing carbohydrate and amino acid metabolism, hydrogen peroxide and malondialdehyde levels, and antioxidant enzyme activity. The third chapter involved greenhouse experiments in which plants grown from seeds treated with priming were monitored over 65 days under salt stress. Parameters such as chlorophyll *a* fluorescence, gas exchange, and markers related to salt tolerance identified in the seeds were analyzed during different phenological stages. The results showed that the cultivars exhibit distinct tolerance mechanisms to osmotic and salt stress. Priming induced positive effects, particularly through the accumulation of proline and sugars, which facilitated osmotic adjustment and mitigated oxidative stress in seeds, seedlings, and adult plants. These effects can be attributed to "priming memory," a phenomenon that allows the recovery of metabolic alterations induced by the initial treatment, resulting in better maintenance of photosynthetic metabolism and reduced impacts of salt stress across different phenological stages. In conclusion, priming is an effective tool for enhancing sorghum tolerance to salt stress, contributing to the development of sustainable strategies that enable cultivation in saline areas. This approach broadens productive potential and agricultural resilience in adverse regions, making it a valuable technique for ensuring food security and sustainable agricultural practices in the face of environmental challenges.

Key words: oxidative stress; climate change; hormopriming; abiotic stress, prolin**e.**

INDICADORES DE IMPACTO

A aplicação do *priming* em sementes de sorgo (*Sorghum bicolor* (L.) Moench) sob condições de estresse salino trouxe impactos notáveis em diversas dimensões: social, tecnológica, econômica e cultural. Socialmente, a técnica contribui para a segurança alimentar em regiões áridas e semiáridas, onde a salinização do solo é uma barreira significativa para a produção agrícola. Ao permitir o cultivo de uma cultura amplamente utilizada como o sorgo, especialmente em comunidades dependentes da agricultura para subsistência, o *priming* ajuda a mitigar os efeitos das mudanças climáticas e das condições adversas do solo. Além disso, ao aumentar a resiliência das plantas a ambientes desafiadores, essa abordagem promove o fortalecimento de pequenos agricultores, especialmente em áreas vulneráveis, promovendo maior autonomia e estabilidade. Do ponto de vista tecnológico, o *priming* representa um avanço significativo nas práticas de tratamento de sementes. Ao utilizar moléculas antioxidantes, como o ácido ascórbico, e fitoreguladores, como o ácido abscísico, a técnica possibilita melhorias no processo germinativo e na tolerância das plantas durante diferentes estágios fenológicos. A memória metabólica induzida pelo priming, evidenciada pelo acúmulo de prolina e açúcares, destaca-se como um mecanismo inovador que permite às plantas ajustarem seu metabolismo osmótico, reduzindo os danos causados pelo estresse salino e oxidativo. Essa abordagem abre caminhos para novos estudos e implementações em outras culturas agrícolas, ampliando o escopo de aplicação da tecnologia. Economicamente, os benefícios são expressivos. O aumento na germinação e no vigor inicial das plântulas gera plantas mais saudáveis e produtivas, resultando em maior rendimento por área cultivada. Esse fator é crucial em regiões onde a disponibilidade de recursos é limitada e a maximização da produtividade se torna indispensável. Além disso, a utilização do priming pode reduzir a necessidade de insumos químicos, como fertilizantes e defensivos, contribuindo para a diminuição de custos de produção. A longo prazo, a técnica também favorece a sustentabilidade econômica, pois culturas mais tolerantes ao estresse salino demandam menos recursos hídricos de alta qualidade, otimizando o uso da água em regiões com escassez hídrica. Culturalmente, o priming desempenha um papel importante ao integrar práticas agrícolas sustentáveis e tecnológicas nas rotinas de agricultores locais. Em regiões onde a agricultura é mais do que uma atividade econômica, mas parte da identidade cultural, o uso de métodos como o priming pode impulsionar uma transição para práticas mais resilientes e conscientes. Além disso, o sucesso da técnica reforça a valorização de culturas adaptadas a condições adversas, como o sorgo, que possuem relevância histórica e cultural em diversas comunidades. Em suma, a aplicação do priming em sementes de sorgo vai além do aumento da tolerância ao estresse salino, gerando impactos positivos em diversas dimensões da sociedade. Ao promover inovação tecnológica, garantir maior segurança alimentar, reduzir custos de produção e valorizar práticas agrícolas tradicionais adaptadas a novas realidades, a técnica se consolida como uma solução sustentável e transformadora, alinhada às necessidades de um mundo em constante mudança.

IMPACT INDEX

The application of priming in sorghum seeds (*Sorghum bicolor* (L.) Moench) under salt stress conditions has brought notable impacts across various dimensions: social, technological, economic, and cultural. Socially, the technique contributes to food security in arid and semi-arid regions, where soil salinization poses a significant barrier to agricultural production. By enabling the cultivation of a widely used crop like sorghum, especially in communities reliant on agriculture for subsistence, priming helps mitigate the effects of climate change and adverse soil conditions. Furthermore, by enhancing plant resilience in challenging environments, this approach empowers small-scale farmers, particularly in vulnerable areas, fostering greater autonomy and stability. From a technological perspective, priming represents a significant advancement in seed treatment practices. By using antioxidant molecules, such as ascorbic acid, and growth regulators, such as abscisic acid, the technique enables improvements in germination processes and plant tolerance during different phenological stages. The metabolic memory induced by priming, evidenced by the accumulation of proline and sugars, stands out as an innovative mechanism that allows plants to adjust their osmotic metabolism, reducing damage caused by salt and oxidative stress. This approach paves the way for further studies and applications in other agricultural crops, broadening the scope of this technology. Economically, the benefits are substantial. Increased germination and initial seedling vigor lead to healthier and more productive plants, resulting in higher yields per cultivated area. This is crucial in regions where resources are limited, and maximizing productivity is essential. Additionally, priming can reduce the need for chemical inputs, such as fertilizers and pesticides, contributing to lower production costs. In the long term, the technique also favors economic sustainability, as crops more tolerant to salt stress require fewer high-quality water resources, optimizing water usage in regions with water scarcity. Culturally, priming plays an important role in integrating sustainable and technological agricultural practices into the routines of local farmers. In regions where agriculture is more than just an economic activity but a part of cultural identity, using methods like priming can drive a transition toward more resilient and conscientious practices. Furthermore, the success of the technique reinforces the value of crops adapted to adverse conditions, such as sorghum, which holds historical and cultural relevance in many communities. In summary, the application of priming in sorghum seeds goes beyond increasing tolerance to salt stress, generating positive impacts across various dimensions of society. By promoting technological innovation, ensuring greater food security, reducing production costs, and valuing traditional agricultural practices adapted to new realities, the technique establishes itself as a sustainable and transformative solution aligned with the needs of a constantly changing world.

SUMMARY

1. INTRODUCTION

Germination is an event that occurs essentially in the presence of water, which plays a key role in providing hydration of tissues, through imbibition, and leads to the recovery of metabolism culminating in the protrusion of the radicle, ending the germination event (Bewley and Black., 2013; Bewley and Black., 2004). However, because this occurs strictly in the presence of water, several factors can affect germination, which can slow down this process or even inhibit it permanently by the time the embryo dies (Çakmakçi et al., 2019). Once it occurs successfully, germination will give rise to a seedling that will colonize the environment and ensure reproductive success. This stage also presents fragility due to stressful environmental factors, such as drought stress, salinity, high radiation and temperature, leading to limitations in the establishment of this seedling that may culminate in lower production yields. Thus, the role of germination is of extreme importance and highly affected by external and internal factors (Ceritoglu et al., 2020).

Several technologies are adopted to improve seedlings tolerance, all aiming to provide conditions for germination and post-germination events to occur adequately, resulting in healthy plants. Among these techniques, seed priming emerges as an efficient and low cost one. Priming consists in the imbibition of seeds, which is interrupted before the protrusion of the radicle (phase II of imbibition curve), followed by drying (preferably fast, to avoid the consumption of reserves) and subsequent storage (Singh et al., 2018; Noreen et al., 2020). This technique can be performed using distinct molecules or solutions. Some time ago, the seed priming was used just for increasing the speed and synchronizing of germination for agronomic purposes. Nowadays, seed priming is being used to recruit a metabolism imposed by exposure to the seeds to a stress simulation. In other words, the priming application in seeds build up a ‘stress memory’, through enhancing different physiological and biochemical mechanisms that can be recruited when they (seeds, seedlings or plants) are exposed to adverse conditions (Aziz et al., 2021). Therefore, the priming is being used for increasing stress tolerance in plants.

One of the main concerned abiotic stressful conditions around the world is the salinity. This condition occurs when there is an increase in the availability of salts (NaCl, KCl and others) in the environment. The increase of these ions in the environment can lead to severe impacts on germination and establishment of seedlings and plant growth and development. The negative impacts of high salinity occur mostly in the dissociation of ions, which may compromise the uptake of water and nutrients by seeds and plants. The impacts imposed by this condition will be guided by (i) the occurrence of ion dissociation, leading to changes in osmotic potential, and consequently water absorption, or (ii) the cytotoxic effects of the accumulation of these ions. Due to the occurrence of salt stress, there are delays in seed germination, reductions in seedlings establishment, and plant´s physiology, i. e, water relations, photosynthesis and nutrition. The salinity culminates, in field conditions, in a poor plant stand and low agronomic indices (Rajabi Dehnavi et al. 2020).

The causes of the salinity are both natural or by anthropomorphic interferences, such as the use of wastewater, increasing agrochemicals using, and misuse of the land. Regions with low rainfall (e.g. semi-arid regions) need not only to deal with drought, but also with the effects of salinity. In these regions the use of crops that better tolerate these conditions is carried out in order to promote food and feed supply, as well as feedstock for fuel or energy production (Nimir et al., 2020). Therefore, the knowing of which crops can stand salinity around the world is essential. and One of these widely used crops is sorghum (*Sorghum bicolor* Moench (L)). Sorghum is a high nutritional species that shows moderate to high tolerance to drought, depending on the phenological stage (McCann et al. 2015; Impa et al. 2019).

This crop is usually cultivated in areas where salinity is a hindrance to food production, such as part of the African continent, Asia, and the Brazilian semiarid (Ibrahim 2016). In these regions, there is a search for genetic materials or techniques that show or increase better responses to salinity and drought. In this way, the use of the priming technique in seeds can be a powerful tool to increase crop´s tolerance to stressful conditions. The priming can also reduce the development time and resources of generating new tolerant cultivars, emerging as a parallel and cheap technique that provides conditions for better establishment of crops, achieving better agricultural results (Shakeri et al., 2017; Çakmakçı and Dallar, 2019).

Thus, this work was conducted based on the hypothesis that the application of priming in sorghum seeds (with water or solutions with hormone or antioxidant) improve seed germination, seedling establishment, and plant growth under salinity conditions through in antioxidant and osmotic adjustments. Three manuscripts (chapters), which the main objectives are described, as follows, compose this work. In the chapter 1, we investigate how the application of priming promotes improvements in the germination of sorghum seeds exposed to high salinity and osmotic stress conditions, using germination parameters for contrasting cultivars for drought tolerance. In the chapter 2, we aimed to identify by which mechanisms the priming improved germination comparing the most discrepant cultivars (higher sensitivity and higher tolerance) to salinity and osmotic conditions. Finally, in the chapter 3, there were evaluated the effect of different types of priming on amino acid, sugar and photosynthetic metabolism of sorghum seedlings and plants grown under doses of NaCl.

2. THEORETICAL FRAMEWORK

2.1. Seed germination and seedling growth – implications for crops´ establishment

Seed germination is a physiological process that begins with the absorption of water by the seed and ends with the protrusion of the radicle (Bewley et al., 2013). Water soaking can be observed as an increase in seed fresh weight due to an increase in water content by the end of germination. Seed water soaking follows a pattern proposed by Bewley and Black (1994) where there are three distinct times following a three-phase pattern. This triphasic pattern is defined as the weight/water content of the seeds varies. In the work of Bewley et al. (2013) there is a definition of these three phases during germination. In the first stage (I) there is the absorption of water by the seed, mainly due to the potential differences between the seed and the germination medium. The third phase (III) is the moment when there is the protrusion of the radicle and an increase in seed weight.

Although initially this process is governed by the difference between the osmotic potentials of the soil and seed, there are intrinsic and abiotic factors that can compromise germination or even cause seed death. The factors that may cause a delay in germination, conditioned to the hormonal balance of orthodox seeds, are initially caused by dormancy, primary or secondary dormancy, and the environmental factors are related to the osmotic potential. The main environmental factors are drought, which can cause an irregular imbibition, that is, the seed starts soaking, however there are no conditions for it to occur properly, or factors that can cause changes in the osmotic potential of the soil solution, caused mainly by ions that compromise the absorption of water by seeds.

We must also consider that until phase II of the soaking curve, seed desiccation can occur without compromising the resumption of soaking. However, if this occurs at the end of phase III, where there is protrusion of the radicle, the seed is unable to undergo desiccation, resulting in embryo death and consequently non-germination of the seed. Among the factors that most impact the establishment of crops, we can highlight that the abiotic factors are the most detrimental.

2.1.1. Seed priming: is it just a technique to synchronize germination?

Seed priming is a worldwide and millenary technique used for promoting improvements and synchronizing germination Sharma et al., 2014. The priming consists in imbibe the seeds until phase II of germination, drying them, storing, following by rehydration (Lutts 2016). Seed priming interferes on duration of germination phases and may cause an anticipation of radicle protrusion, through the regulation of metabolic processes that are responsible for the initial phases of germination (Ibrahim et al., 2016. This technique leads to several improvements, especially in reducing the imbibition time, leading to increases in the production of metabolites, such as amino acids, and synthesis of enzymes and proteins that may promote DNA repair or regulation of redox metabolism (Wardah et al., 2019).

The application of this technique has shown to be very efficient in promoting tolerance to unfavorable environmental conditions to germination, such as drought, salt and heat stress. In addition, the use of priming in seeds, besides being safe, can present itself as a low-cost alternative for crop production (Ibrahim, 2016). This technique results in increasing speed and uniformity of germination as well as improve seed vigor and seedling establishment under stressful conditions (Gupta et al., 2008, Sharma et al., 2014; Patade et al., 2009, Bewley et al., 2013).

The application of the technique is usually in aqueous solutions, followed by the rapid drying of these seeds soon after exposure to rehydration. It is important to note that although it is performed with the use of aqueous solutions there are techniques that use solid substances to perform the technique. The type of priming to be applied is an important step to obtain effective responses to the various unfavorable environmental conditions faced by seeds and plants. The most commonly used techniques are the hydropriming (water as a medium of imbibition), halopriming (salt solutions such as NaCl or MgCl), osmopriming (use of solutions with different osmotic potentials, usually induced by polyethylene glycol - PEG) or hormopriming (use of growth regulators) (Karadag et al., 2017; Hassini et al., 2017; Roychoudhuryet al., 2016; Salama et al., 2015; Nakaune et al., 2012).

The effects of priming can be observed during all phenological stages of plants (Huang et al., 2016). In seeds, the effects may be observed that will promote faster imbibition, ensuring the integrity of the macrostructures and membranes, preventing damage that compromises germination success. The application of priming allows a rapid absorption of water, ensuring greater uniformity of germination and also the recovery of metabolism more efficiently, mitigating deleterious effects such as the formation of reactive oxygen species (ROS) that may compromise the germination and generation of normal seedlings (Talukdar, 2012; Kumar et al., 2014).

Thus, when primed, seeds may exhibit conditions that trigger modifications of metabolism pathways, making them more able to maintain their development during unfavorable conditions. In the primed state, seeds can exhibit multiple epigenetic changes, especially up-regulation of stress-responsive transcription factors, thereby improving germination and seedling establishment compared to untreated seeds and promoting increases in productivity (Sharma et al., 2014; Jisha et al., 2013; Farooqet al., 2009; Bruce et al., 2007).

In seedlings and plants, the effects can be observed when analyzing the benefits in maintaining redox homeostasis and in improvements in photosynthetic metabolism (Fujita et al., 2013). During these phenological stages, it can be observed that there are improvements that go through the inhibition of cytotoxic effects, besides the maintenance in the repair of photosystems and regulation of stomatal opening. The application of the priming technique besides these adjustments, also promotes the *de novo* synthesis of proteins and also the accumulation of hormones in *Vigna radiata* and *Arabidopsis* (Jisha et al., 2013; Rajjou et al., 2012; Zhou et al., 2012), a higher content of sugars and proline has been observed in rice by Karalija and Selovic (2018) and Gul et al. (2020) and Nouairi (2019) have observed an improvement in photosynthetic rate in rice and fava beans. All these findings leading us to believe that the application of priming could be highly effective in promote the benefits expected.

2.1.2. Salinity and its effects on germination, seedling establishment and plant growth

Most of the agricultural areas are subject to salinization that is conceptualized by the excessive deposition and accumulation of mineral salts in the soil (FAO, 2011; Fedoroff et al., 2010). Salinity is a current problem, in a growing scenario, and that demands high investments in its repair. The deposition of ions in the soil can occur in natural ways, due to leaching of rocks or by the indiscriminate use of fertilizers and wastewater containing high concentrations of ions such as KCl or NaCl (Fedoroff et al., 2010). The effects of salinity can occur in two ways, (i) by the osmotic effect, caused by the decrease in osmotic potential; and (ii) by the ionic effect, due to the dissociation of ions leading to severe cytotoxic effects (Li et al., 2020).

Under stressful environmental conditions, such as drought and salinity, there is the perception of these conditions mainly by systemic signaling, and one of the first responses is the accumulation of the phytohormone abscisic acid (ABA). In seeds, this accumulation may inhibit germination, due to the imposition of a secondary dormancy, under salinity conditions in the soil, germination is compromised as the deposition of salts advances. Low concentrations of salts in the soil tend to induce a state of dormancy in the seed. Whereas if an increase in the concentration of salts in the soil solution occurs, germination is inhibited, culminating in a lower percentage of germination by healthy seeds (Khan et al., 2006; Thiam et al., 2013).

In seedlings and plants, this may lead to a decrease in stomatal conductance, which is one of the conditions responsible for the increased production of ROS. Depending on the extent and duration of the stress condition, the increasing ROS formation may cause oxidative stress, that is measured by the oxidative damage (Sharma et al., 2012; Huang et al., 2016). In salinity conditions, it is possible to observe in plants an accumulation of Na+ in leaves. The accumulation of this ion leads to severe damage that compromises the development and yield of crops.

The main damage that can be observed and the most deleterious could be observed in seeds, seedlings and full growth plants. Seeds are affected by salinity through the decrease in the osmotic potential, delaying the imbibition of water, inhibiting the germination, or even by the damage caused by the dissociation of ion, these conditions take place cause these phenomena causes toxicity and could lead the seeds to death (Li et al., 2020). In seedlings and plant, since growth are dependent of photosynthesis, the effects are related by the alteration in water and nutrients uptake, and gas exchanges and oxidative damage. In plants are a row of effects that comprises the metabolism, that culminated in reduction of biomass and metabolic impairments, the main observed effect are i- dehydration of membranes reducing the CO_2 permeability, ii-the effects in water and nutrient uptakes, that could impact the photosynthesis and other metabolic processes, iii- reduction in the CO_2 supply by the stomatal closure, this condition lead to an condition of oxidative damage, since there is an excess of energy captured by the light harvest complex that was not conducted to the carboxylation process, this energy could interact with oxygen and generate reactive oxygen species. These deleterious effects on are related to photosynthetic efficiency (decrease in the efficiency of FSII), and decrease in the quantum efficiency of FSII (Fv/Fm), as well as photoinhibition. An increase in ROS formation due to the detour of electrons from the electron transport chain (ETC), the formation of these molecules when above levels that allow their elimination can lead to structural damage (membrane lipid peroxidation by H_2O_2) and DNA damage (Yang et al., 2014; Murata et al., 2007).

The application of priming is able to promote modifications in the way plants circumvent this scenario. Firstly, there is a decrease in imbibition and germination time, this effect associated with priming allows the rapid emergence of the seedlings allows for a rapid perception of the environment, leading to metabolic changes and adjustments that will make it more able to pass through the imposed adverse condition (Wardah et al., 2019). Among these, one can point out improvements through morphological modifications, (phenotypic plasticity), physiological (such as membrane stability through better osmotic adjustment), in addition to changes in biochemical and molecular mechanisms (accumulation of proline, auxins, ABA and ethylene, stress-sensitive proteins, transcription factors and secondary messengers). All these changes that lead to plant adaptation are objectives in breeding programs, and when they can be observed through the application of priming, this makes the technique even more relevant, since there is a considerable decrease in the time required in breeding through the application of this technique (Langeroodi and Noora, 2017; Mohammadi et al., 2014). Due to application of the technique, there is a phenomenon called priming memory, this type of memory is a set of modification imposed by the priming procedure that allow the seeds and plants to recovery a arrange of metabolic modifications, induced by the priming, when they face stress conditions.

The evocation of this memory enable the seeds to acclimatize to harsh condition to germination, by recruiting osmoprotective molecules, as well as antioxidants. Any plants can “access” this memory by recovering biochemical and physiological adaptative process to overcome the stressful conditions (Hameed et al., 2021; Valivand et al., 2019).

2.1.3. Sorghum Varieties: socio-economic importance and stress tolerance

Sorghum (*Sorghum bicolor* (L.) Moench), the family Poaceae, subfamily Panicoidea, is the fifth largest grain crop on the world and due to its economic importance, sorghum is grown worldwide, especially in tropical regions, both for human and animal food, as well as for the production of biomass for biofuels (Awika et al., 2004; Queiroz et al., 2011; Fita et al., 2015). The sorghum culture is divided into two areas; one area is concentrated in African and Asian countries, where production occurs in a traditional way, as subsistence agriculture, without technological apparatus, having low productivity. In this region, the production is focused on human food. The second region where the crop prevails includes some developed countries in America, where a large-scale mechanized system is employed, and reaching high productivity.

In these regions, the production is destined for animal feed. The largest producers of sorghum are the United States, Mexico and Nigeria and the main exporters are the United States, Australia and Argentina (FAOSTAT, 2014; Sanders et al., 2019).

Due to high versatility of growing, the main cultivation regions of the species are in tropical and subtropical areas. It is liable, this way, to find this crop in some cultivation areas in which the environmental and soil conditions are often not profitable to seed germination, seedling establishment, and growth and development, which can culminate in loss of productivity (Fita et al., 2015; Kebede et al, 2001). Therefore, the species could be considered tolerant to many abiotic stressful conditions.

Although sorghum is noted as a crop with moderate tolerance to salinity, germination and seedling establishment are severely affected by the excess of inorganic ions in the soil (Dias et al., 2010). This crop is an important source of supplies in regions where there are high concentrations of salts in the soil, as well as the practice of irrigation with water from salt-contaminated reservoirs is an available resource as in semiarid regions of the African continent and in Brazil (Hassanein et al., 2010; El Naim et al., 2012, Sun et al 2014; Guimaraes et al., 2016). Even among the varieties considered tolerant to drought, it is important to investigate the degree of tolerance to salinity, once sorghum is usually cultivated in regions with high concentrations of salt.

In this work we used the varieties of sorghum, the adoption of these cultivars is based on their drought tolerance or sensibility. We selected two drought-tolerant cultivars (BRS-310 and DKB 540) and two cultivars susceptible to drought (DOW 50A10 and BRS-332) to evaluate the distinct effects of salinity. The cultivars BRS-310 and 332 are developed by the Embrapa, both are highly cultivated in Brazil. The cultivars DOW 50A10 and DKB 540 have been developed by Dow and Dekalb respectively. The selection of these cultivars are based on the necessity of cultivars that provide to us biochemical markers that allow us to access how different varieties responds to the effects drought and salinity stress.

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CHAPTER I

PRIMING ON SORGHUM SEEDS UNDER SALINITY AND OSMOTIC TREATMENTS: ARE THE SEEDS CAPABLE TO GERMINATE UNDER HARSH CONDITIONS?

ABSTRACT

Seed priming is a widely used technique to improve seed germination. However, it has been used for increasing seed tolerance to harsh conditions. Seeds are highly susceptible to variations in osmotic potential, since imbibition is a crucial process for germination. If the osmotic potential changes, severe damage can occur. Osmotic and salt stresses present as a major problem, since they cause serious losses in crops´ establishment. This work aimed to investigate the efficiency of hydropriming and priming with different molecules (ascorbic acid (AsA) and abscisic acid (ABA)) in varieties of sorghum seeds after priming application and under salinity and osmotic conditions. The main question addressed is if the type of priming can improve germination in drought-tolerant and sensitive varieties of sorghum under salt and osmotic conditions. Some of the observed changes are related to the application of priming, it is possible to observe that there are benefits from the application of priming, especially with the molecules AsA and ABA, in the seeds of the sensitive cultivar (BRS-332). This benefit can be found for both osmotic and salt stress conditions. The tolerant cultivars (BRS-310 and DKB 540) were efficient under both conditions when submitted to priming and hydropriming, germination values were high even under the highest NaCl doses and lowest osmotic potentials. Seeds of the cultivar DOW 50A10 (sensitive the lowest germination rate, and oscillations in the T50 and GSI parameters, indicating compromises that could not be bypassed by priming. Overall, the results are optimistic in clarifying the effects of different types of priming on germination, since the results obtained especially for the sensitive cultivar, which had a similar performance to tolerant cultivars when subjected to priming.

Key words: Ascorbic acid; Abscisic acid; germination; *Sorghum bicolor*; PEG6000; NaCl.

1. INTRODUCTION

The impacts of salinity in crops is worrying even in high tolerant species like sorghum (Krishnamurthy et al. 2007), the world's fifth largest cereal crop, present in the dietary of more than 500 million people (FAOSTAT, 2014). Sorghum is an important staple crop for more than half a billion people, mainly in developing countries in the semi-arid and arid tropics, which are prone to water scarcity. It provides nutrition that is rich in protein, fiber, and gluten-free (McCann et al. 2015; Impa et al. 2019). In addition to human nutrition, it is being used as a source of feedstock for bioethanol production (Mathur et al. 2017). Many studies reveal that the tolerance of sorghum genotypes to salinity must be monitored since seed germination and seedling establishment (Krishnamurthy et al. 2007).

Since high salinity and osmotic stress can lead to severe impacts that negatively affect crop production, several efforts have been made to improve low-cost techniques to achieve efficient results in promoting better seed germination. One of these techniques is the seed priming, a low cost and ‘ecologically friendly’ technique to improve seed germination (Ibrahim 2016). During seed priming, several physicochemical alterations can occur that can modify the characteristics of the plant, improving the physiological activity of the embryo and the future plant (Kaur and Gupta 2018).

The incorporation of growth regulators and hormones in the priming technique is a promising alternative, since it is possible inducing remodeling pathways towards salinity tolerance. Two molecules that, when applied as conditioners, show promising results are ascorbic acid (AsA) and abscisic acid (ABA). AsA is a potent antioxidant, and has an efficient effect in combating stress damage, and some studies using exogenous applications of AsA have suggested that it can counteract the adverse effects of salinity (Singh et al., 2018; Noreen et al., 2020). Under salinity conditions, ABA has been shown to play an important role in improving salinity tolerance through enhanced germination and growth performance of different crops (Gurmani et al., 2011). The metabolic and molecular changes by the ABA and AsA signaling lead to an accumulation of osmolytes and expression of transcription factors that allowed the seeds to circumvent the unfavorable condition (Nadarajah et al., 2020; Alam et al., 2019). However, besides being promising, the use of AsA and ABA to induce cross-tolerance in seeds by priming application is still underexploited and, consequently, poorly understood.

This way, we investigate in this work the effects of primed-sorghum seeds (with water – hydropriming; and with ascorbic acid (AsA) or ABA) subjected to salinity and simulated osmotic stress. We hypothesize that priming with AsA and ABA, can improve seed germination and uniformity even in high concentration of salt, in relation to non-primed seeds.

It is also expected that contrasting drought-tolerant sorghum varieties can germinate differently on salinity or osmotic conditions. It could give to us cues about the improvements on germination capacity upon priming treatments when exposed to osmotic stressful conditions.

2. MATERIAL AND METHODS

2.1. Plant material

Seeds of four *Sorghum bicolor* (L.) Moench cultivars were used in this experiment. They differ in tolerance and susceptibility to water deficit: BRS 310 and DKB 540 are drought-tolerant; BRS 332 and DOW 50A10 are sensitive. All material was provided by Embrapa Maize and Sorghum, Brazil.

2.1.1. Priming procedure

The priming technique was set according to Ibrahim (2016), considering a imbibition step until phase II (with water or solutions), a drying step to reach the initial weight, a short-time storing step, followed by germination. The conditioners concentrations were previously determined according to pre-tests and based on doses found in the literature. The ABA solution was performed following Vieira et al. (2017). ABA was diluted in 1N KOH solution (the content used to the complete dissolution of ABA was approximately 0,2ml), in dark conditions. The volume was adjusted to 400ml and the pH was adjusted for 7.0, if needed.

The AsA solution was performed in the dark, with dilution in deionized water. The seeds were imbibed using solutions containing the phytoregulator ABA or AsA, or just water, for a period of 8 hours. The time of 8h-imbibition was set in a previous experiment to estimate the imbibition curve and matched the half of phase II. The doses of hormones and ascorbic acid were set at 100 µM, according to the results obtained in the pre-test (the concentration that did not reduce seed germination percentage and velocity). Then the seeds were dried in a forced circulation oven for 12 hours at 30°C, until achieve the initial water content. After this, the seeds were stored for 3-7 days maximum at room-controlled temperature (25 ºC) until the germination experiments.

2.1.2. Experimental conditions

The experiment was conducted in the Laboratory of Plant Growth and Development (LCDP) of the Federal University of Lavras (Lavras-MG, Brazil). An individual experiment was conducted for each cultivar, in each experimental condition (priming treatments vs salinity and osmotic conditions). The seeds were exposed to priming with the signaling molecules (ABA and AsA, as well as to hydropriming (for insulating priming effects without conditioners) in the times of imbibition and drying as mentioned. After priming procedure, the seeds were exposed to the experimental conditions: five NaCl doses (0, 60, 120, 180 and 240) and the respective osmotic potentials (-2.93, -5.86, -8.8, and -11.73 MPa), induced with PEG6000 (polyethylene glycol). The osmotic potential of the solutions was calculated based on equation of Van´t Hoff (Van´t Hoff 1887). Thus, the experiment was designed in a 5x4 factorial scheme (5 doses of NaCl or PEG6000 *vs* 4 priming conditions) for each cultivar.

2.1.3. Germination parameters

To obtain the germination parameters, the seeds were placed in Petri dishes between a double layer of germination paper, moistened with 10ml of solution containing NaCl or PEG-6000. There were used 4 repetitions with 25 seeds in a germination chamber at 12h photoperiod at 40µmol photons m^{-2} s^{-1} at 25º C. Germination was observed every eight hours for a period of 48 hours and the criterion was 2mm of radicle protrusion. The germination percentage (G) was used to build the cumulative germination curve. The germination speed index (GVI) was calculated according to Maguire (1962). The median germination time (T50), that corresponds to the time required for 50% of the seed lot to germinate, was calculated according to Farooq *et al.*, (2005).

2.1.4. Statistical analysis

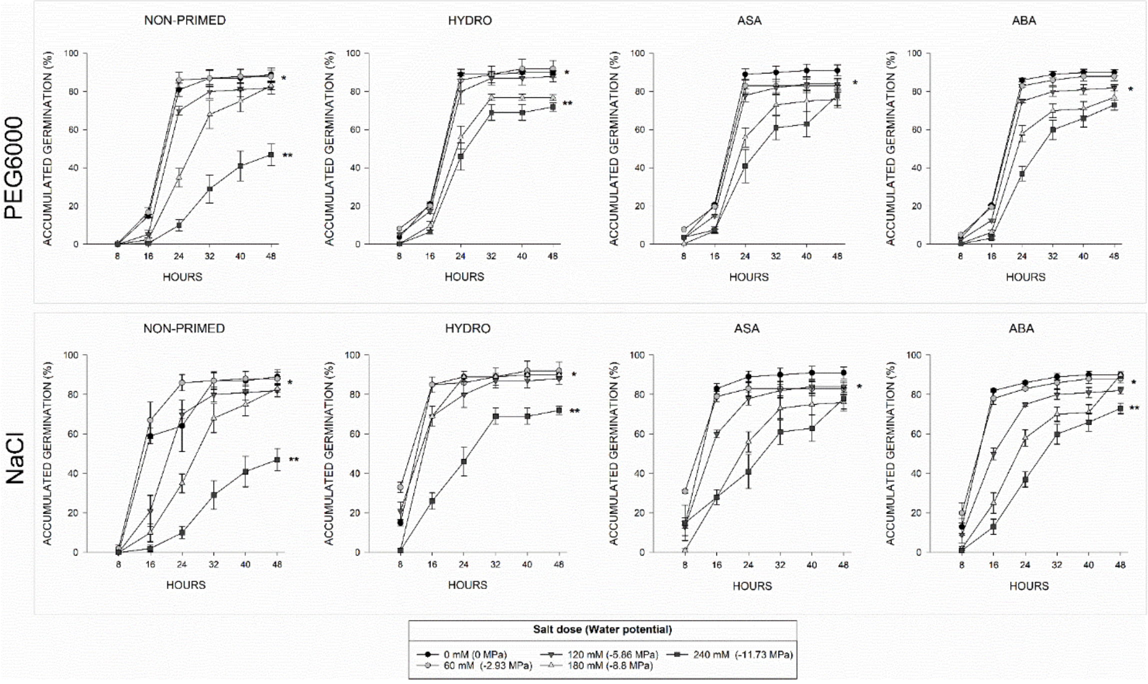
The statistical analysis was performed with the software Rbio (Bhering, 2017). Data was subjected to two-way ANOVA, the Tukey means test was performed in case of normal distribution. The Principal Components Analysis was performed using the software R (stats and factoextra packages).

For a better understanding of the effect of the conditions, doses and osmotic potentials on the analyzed variables we performed principal component analysis (PCA) comparing the cultivars and the conditions, and comparing the effects of NaCl doses and osmotic potentials simulated by PEG into the cultivars. The variables analyzed were germination percentage for the cultivars, and the effects of the doses and potentials within the variable G and T50.

3. RESULTS

The seeds of the cultivar BRS-310 showed higher germination when subjected to hydropriming or AsA in comparison to the dry seeds. It is possible to observe (Fig. 1.1) that the primed seeds presented enhanced germinability when submitted to NaCl than the PEG6000 solutions, even at lower osmotic potentials. Primed seeds presented higher germination during the first 8 hours of evaluation, with the lowest percentages of germination at this period being observed at the highest doses of salt (180 and 240 mM respectively).

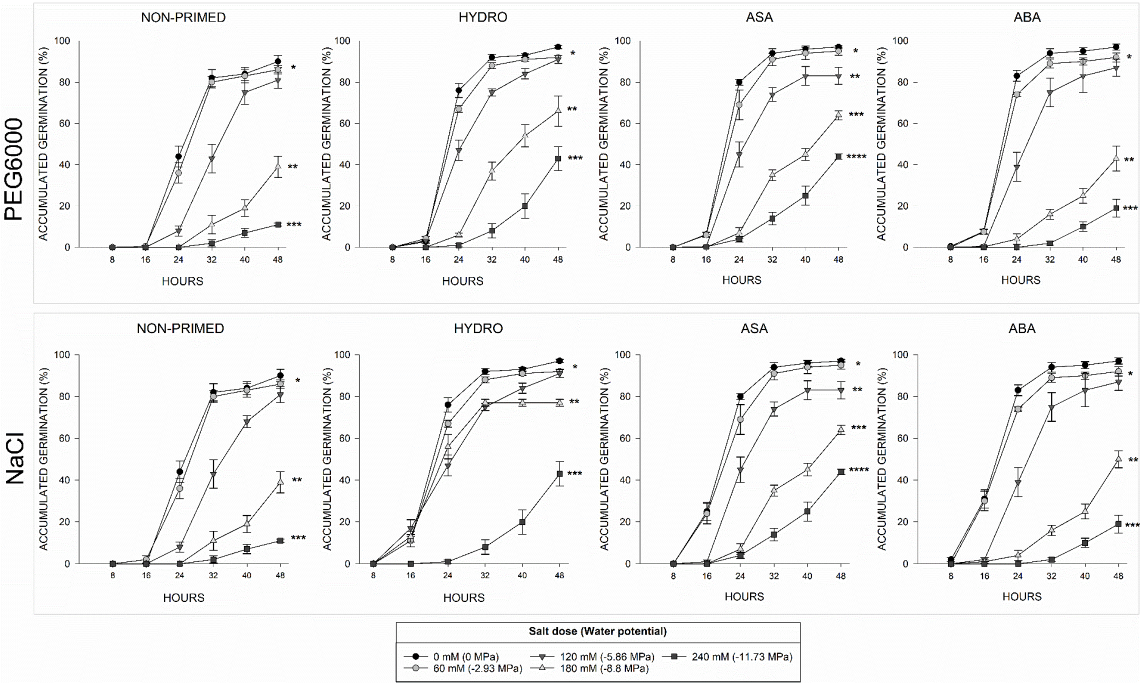
Figure 1.1 - Accumulated germination of the cultivar BRS-310 that was subjected to PEG-6000 solutions at respective osmotic potentials -2.93, -5.86, -8.8, and -11.73, and to solutions of water with 60, 120, 180, and 240 mM NaCl. Values represents means ±Standard error (*n=4)* Asterisks represents the statistical differences at P<0.05 (Tukey test) at final germination percentage.



Source: Ferreira (2022)

Under osmotic conditions, BRS-332 presented a higher sensitivity at PEG6000 for germination when compared to salt in the period between 8h and 16h of evaluation (Fig. 1.2). The primed seeds, besides increased germinability than the dry seeds, still presented a lower germination at lower osmotic potentials. However, under saline conditions, the hydropriming was able to provide a similar germinative percentage up to 180mM.

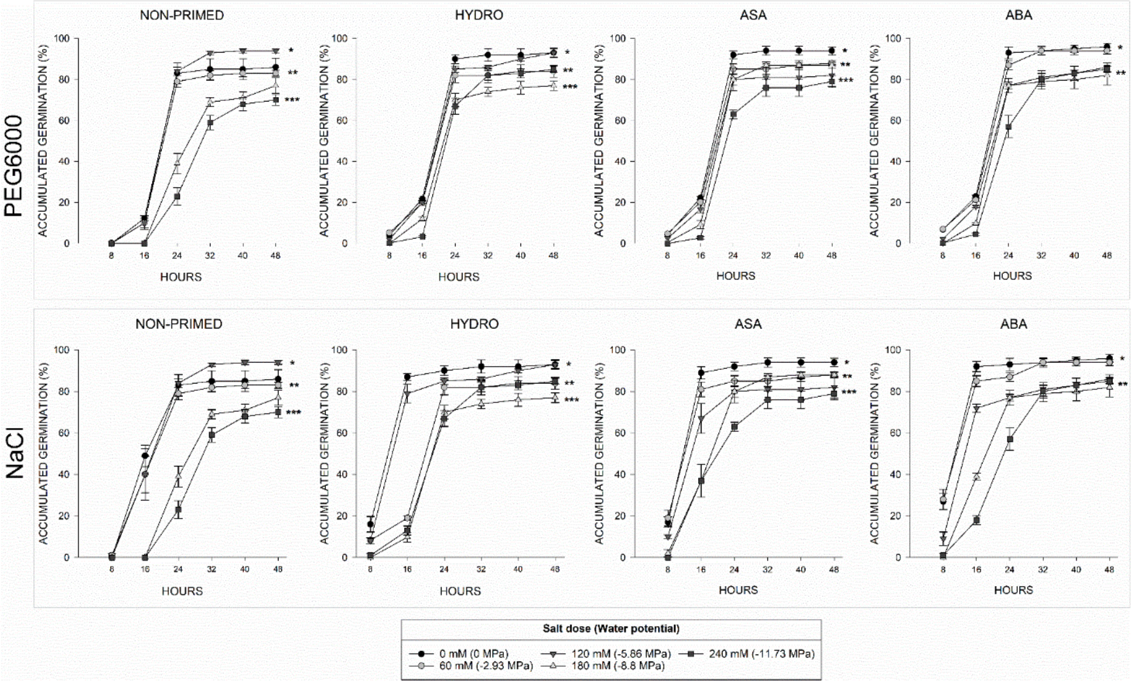
Figure 1.2 - Accumulated germination of the cultivar BRS-332 that was subjected to PEG-6000 solutions at respective osmotic potentials -2.93, -5.86, -8.8, and -11.73, and to solutions of water with 60, 120, 180, and 240 mM NaCl. Values represents means ±Standard error (*n=4)* Asterisks represents the statistical differences at P<0.05 (Tukey test) at final germination percentage.



Source: Ferreira (2022)

Similar to BRS-310 and BRS-332, the seeds of the cultivar DKB 540 had higher germination during the initial periods of evaluation when submitted to priming and at saline conditions (Fig. 1.3). When compared to PEG, the seeds submitted to salt solutions exhibited enhanced germinability between 8h and 16h, however, at 8h the cumulative germination of the seeds submitted to priming was higher than the dry seeds.

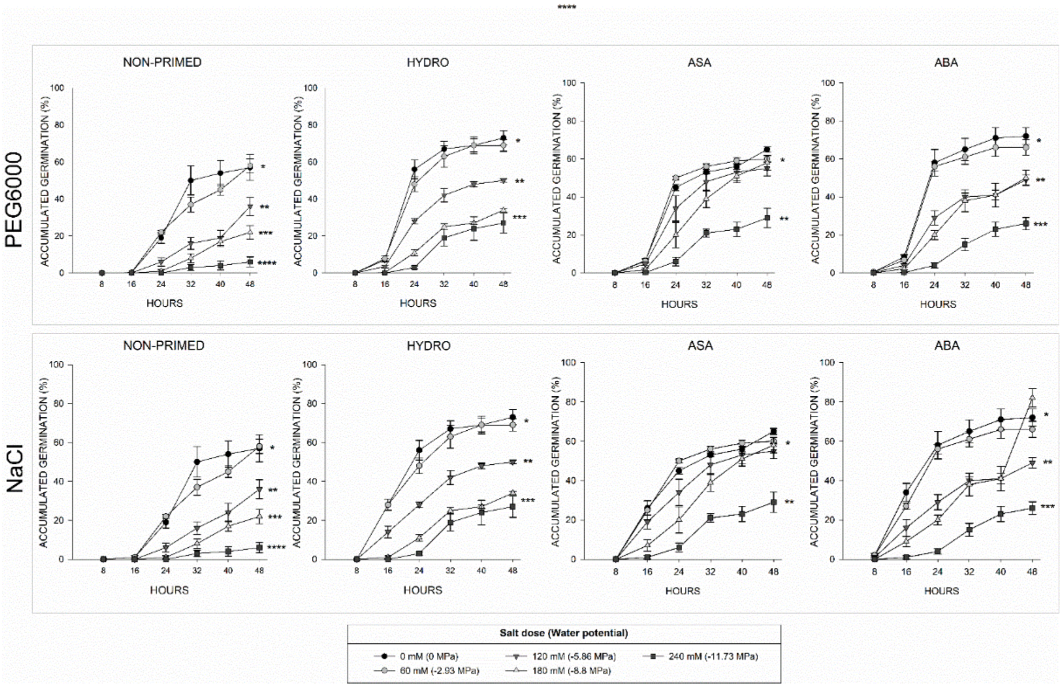
Figure 1.3 - Accumulated germination of the cultivar DKB 540 that was subjected to PEG-6000 solutions at respective osmotic potentials -2.93, -5.86, -8.8, and -11.73, and to solutions of water with 60, 120, 180, and 240 mM NaCl. Values represents means ±Standard error (*n=4)* Asterisks represents the statistical differences at P<0.05 (Tukey test) at final germination percentage.



Source: Ferreira (2022)

Under both conditions (PEG and NaCl), the seeds of the cultivar DOW50A10 presented asynchrony of germination when compared to the other cultivars, achieving approximately 50% of germination at 24-32 hours and lowest germination among the evaluated cultivars, even when submitted to priming (Fig. 1.4). For all cultivars, maximum germination was reached close to 32 hours after evaluation, timing in which BRS-310, BRS-332 and DKB 540 even in NaCl or PEG treatments had the germination percentage stabilization.

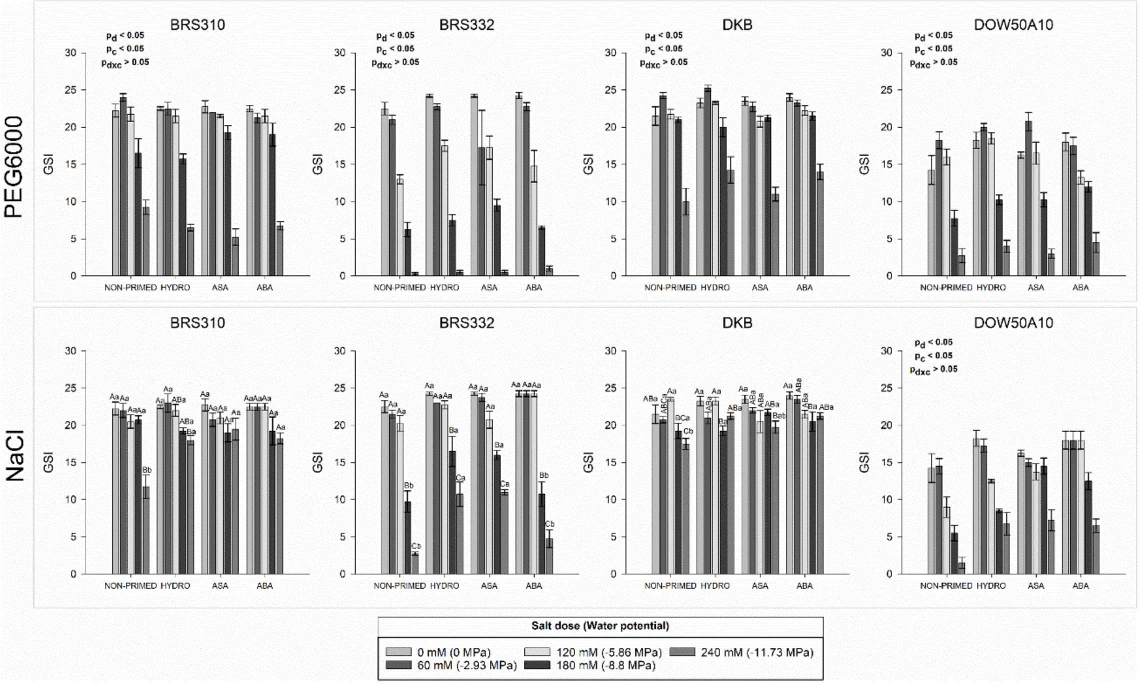
Figure 1.4- Accumulated germination of the cultivar DOW 50A10 that was subjected to PEG-6000 solutions at respective osmotic potentials -2.93, -5.86, -8.8, and -11.73, and to solutions of water with 60, 120, 180, and 240 mM NaCl. Values represents means ±Standard error (*n=4*) Asterisks represents the statistical differences at P<0.05 (Tukey test) at final germination percentage.



Source: Ferreira (2022)

When subjected to PEG, except for DOW 50A10, which showed uninform germination and the lowest and GSI under the control conditions (0 mM). In the lowest osmotic potentials, the two cultivars BRS (332 and 310) and DKB 540, showed similar GSI values. The lowest values for GSI were observed for the lowest osmotic potentials, even within the conditioners, with BRS-332 showing the lowest values when subjected to the -11.73 potential (Fig. 1.5).

Figure 1.5- Germination Speed Index of four Sorghum cultivars (BRS-310, BRS-332, DKB 450 and DOW 50A10) subjected to PEG-6000 at respective osmotic potentials -2.93, -5.86, -8.8, and -11.73, and water solutions with 60, 120, 180, and 240 mM NaCl. Comparison by upper case



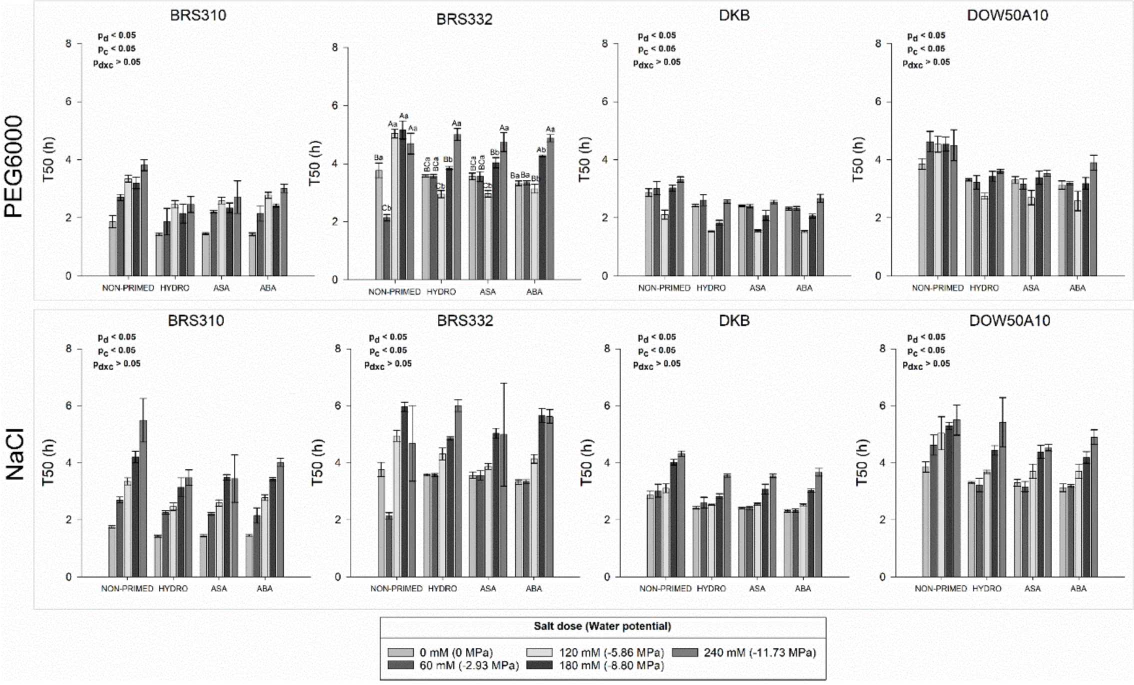
Source: Ferreira (2022)

letter of doses within conditioning, comparison by lower case letter of doses within conditioning or osmotic potential. Values represents means ±Standard error (*n=4)* Asterisks represents the statistical difference at P<0.05 (Tukey)

The cultivar DOW 50A10 showed similar responses to the BRS-332 when subjected to salt doses. BRS-332 the drought sensitive cultivar, presented a better GSI when compared to the osmotic stress condition simulated by PEG. The other cultivars did not differ statistically, although it is possible to observe a higher GSI for BRS-310 and DKB 540 when submitted to salinity conditions.

The average germination time (T50) was negatively affected in seeds subjected to PEG-6000. In this condition the drought susceptible cultivars took longer to establish 50% of germination, with BRS-332 showing an average time of approximately 5h and DOW 50A10 an average time of 4.5h. Meanwhile, BRS-310 and DKB 540 in these same conditions showed similar T50, around 2h at higher water potentials and values close to 3h at lower potentials (Fig. 1.6).

Figure 1.6- Mean germination time (T50) of four Sorghum cultivars (BRS-310, BRS-332, DKB 450 and DOW 50A10) subjected to PEG-6000 at respective osmotic potentials -2.93, -5.86, -8.8, and -11.73, and water solutions with 60, 120, 180, and 240 mM NaCl. Comparison by upper case letter of doses within conditioning, comparison by lower case letter of doses within conditioning or osmotic potential. Values represents means ±Standard error (*n=4)* Asterisks represents the statistical difference at P<0.05 (Tukey)



Source: Ferreira (2022)

When analyzing these cultivars under salinity conditions, DKB showed a lower T50, reaching it around 3h in all treatments. The drought susceptible cultivars had a T50 near 4h in the lower doses of salt, however, when the dose was increased this T50 reached near 6h, both for BRS-332 and DOW50A10.

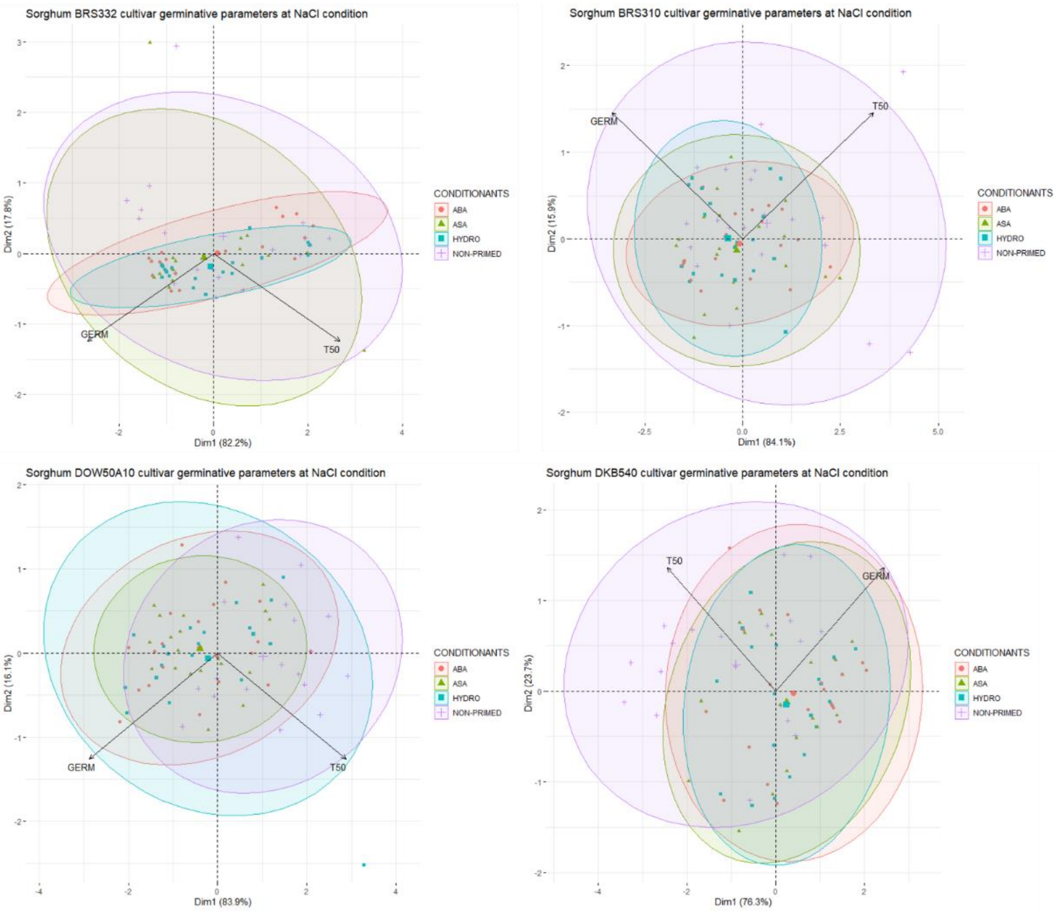
Non-primed seeds are more dispersal then the seeds subjected to priming, regardless the type of priming, indicating that the use of priming are responsible to promote a better grouping close to the arrow who evaluate the final germination (Fig. 1.7 and 1.8). This distance of the germination value and the proximity of T50 arrow indicate that these parameters are highly affected by the use of priming, even for the salinity conditions or the simulated osmotic stress through PEG-6000. For the cultivars BRS-332 and BRS-310, the hydropriming and the priming with ABA showed higher correlation.

It is possible to observe that the cultivar BRS-332 had a negative correlation between G% and T50; this indicates that there is a lower germination and a considerable increase in T50, because this parameter has increased due to a longer period to occur the germination of

50% of the seeds, explained by axis 1 (82.2%). Hydropriming and ABA had closer proximity to axis 1. The DOW cultivar also showed a negative correlation between germination percentage and T50, with lower values being observed for the G variable. ASA and ABA have a higher proximity to axis 1 (83.9%) and are the priming types that best represent the results.

For the water deficit tolerant cultivars (BRS-310 and DKB 540), it could be observed that BRS-310 has the greater results for ASA and ABA, although they also had a negative correlation, these priming types are closer to axis 1 (85.1% of the data). Similar to BRS-310, DKB 540 showed a negative correlation between T50 and germination percentage; the primed seeds had higher values for G and lower values for T50.

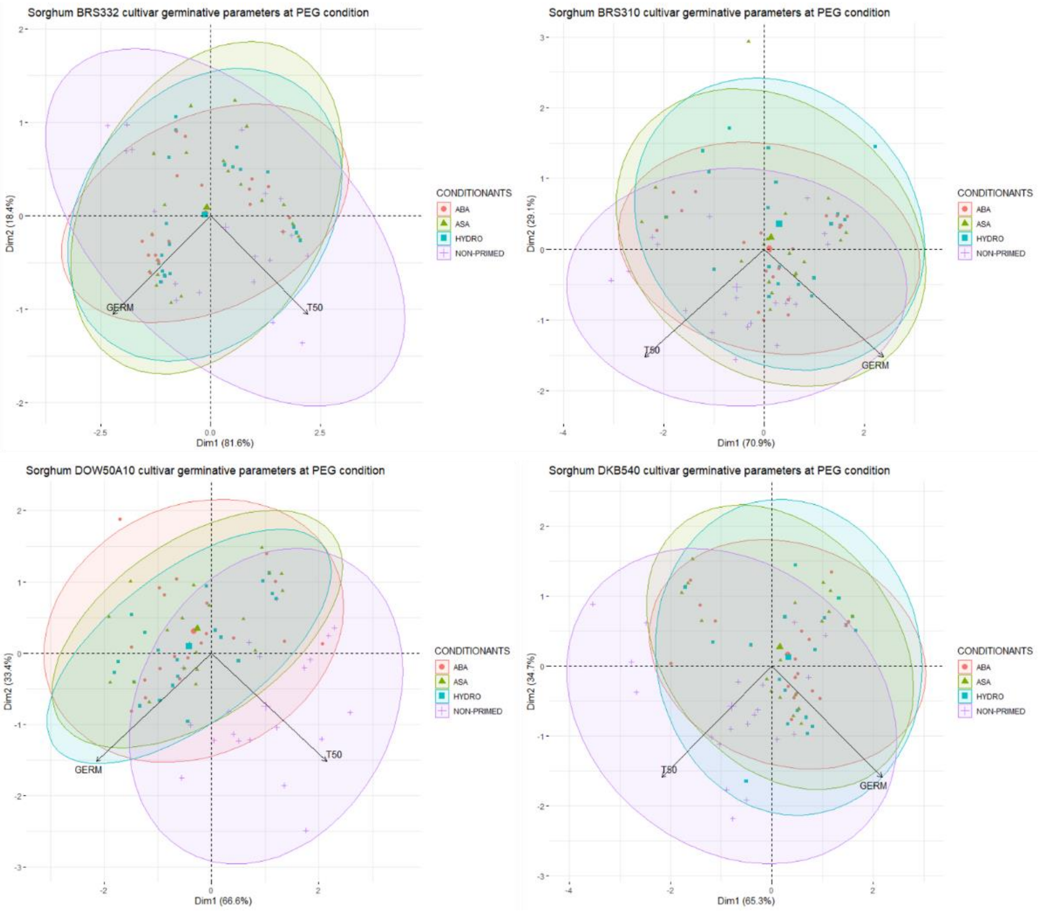
Figure 1.7- Principal components analyses of four cultivars of sorghum (BRS-310, BRS-332, DKB 450 and DOW50A10) primed with 100µM of AsA, ABA, hydroprimed and non-primed subjected to water solutions contend 60, 120, 180, and 240 mM NaCl.



Source: Ferreira (2022)

Under the treatments with PEG6000 (Fig. 8), it is possible to observe the representations for the different cultivars. The cultivars 332 and DOW showed a negative correlation between the variables evaluated under osmotic stress condition, occurring negative effects on germination, leading to the occurrence of an increase in the variable T50. For both cultivars, it can be observed that there is a greater delay in germination for non-primed seeds. The cultivars BRS-310 and DKB showed a similar response. The priming with ASA and ABA, as well as hydropriming promoted a higher G. It is possible to observe higher values for T50 in the non-primed seeds of both cultivars.

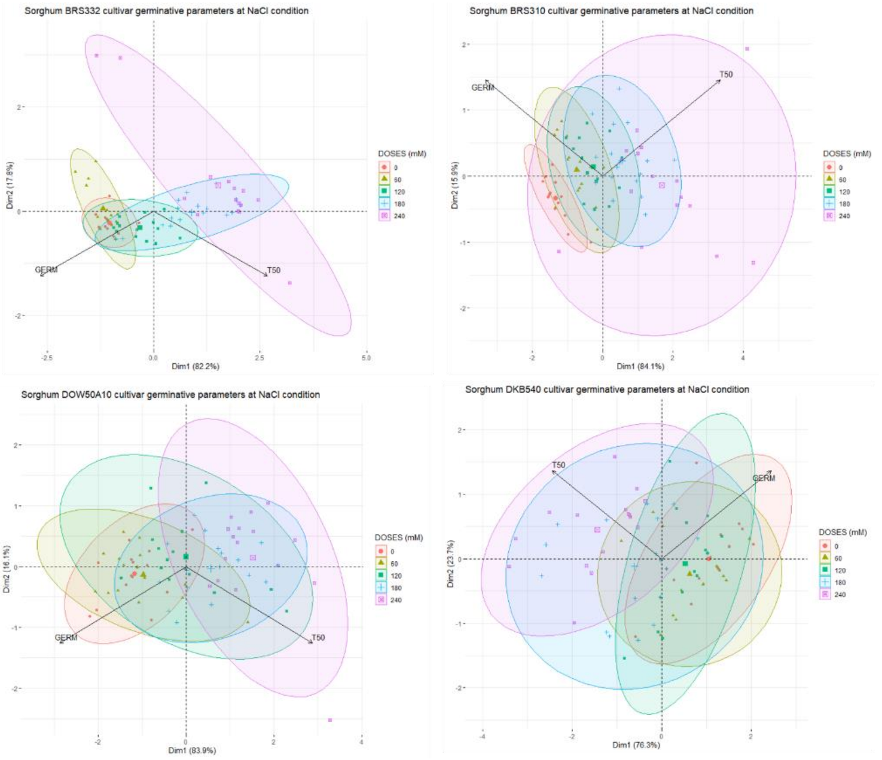
Figure 1.8- Principal components analyses of the effects of PEG-6000 simulated osmotic potentials (-2.93, -5.86, -8.8, and -11.73) on T50 and germination percentage of four cultivars of sorghum (BRS-310, BRS-332, DKB 450 and DOW50A10) primed with 100µM of AsA, ABA, hydroprimed and non-primed.



Source: Ferreira (2022)

Figures 9 and 10 show the principal component analysis of the effects of salt doses and the osmotic potentials for sorghum cultivars regarding the variables G and T50. In general, the higher doses were responsible for providing a greater proximity of the T50 values.For the cultivars, when submitted to salt stress (Fig. 1.9), it was possible to observe the lowest values for germination percentage and a significant increase for the cultivars BRS-332 and DOW, especially above the 120mM NaCl dose, negatively affecting these two cultivars. DKB and BRS-310 showed an increase in T50 for the dose of 240mM of NaCl, and there were better responses for the variable germination percentage for the doses up to 180mM, especially for the DKB540.

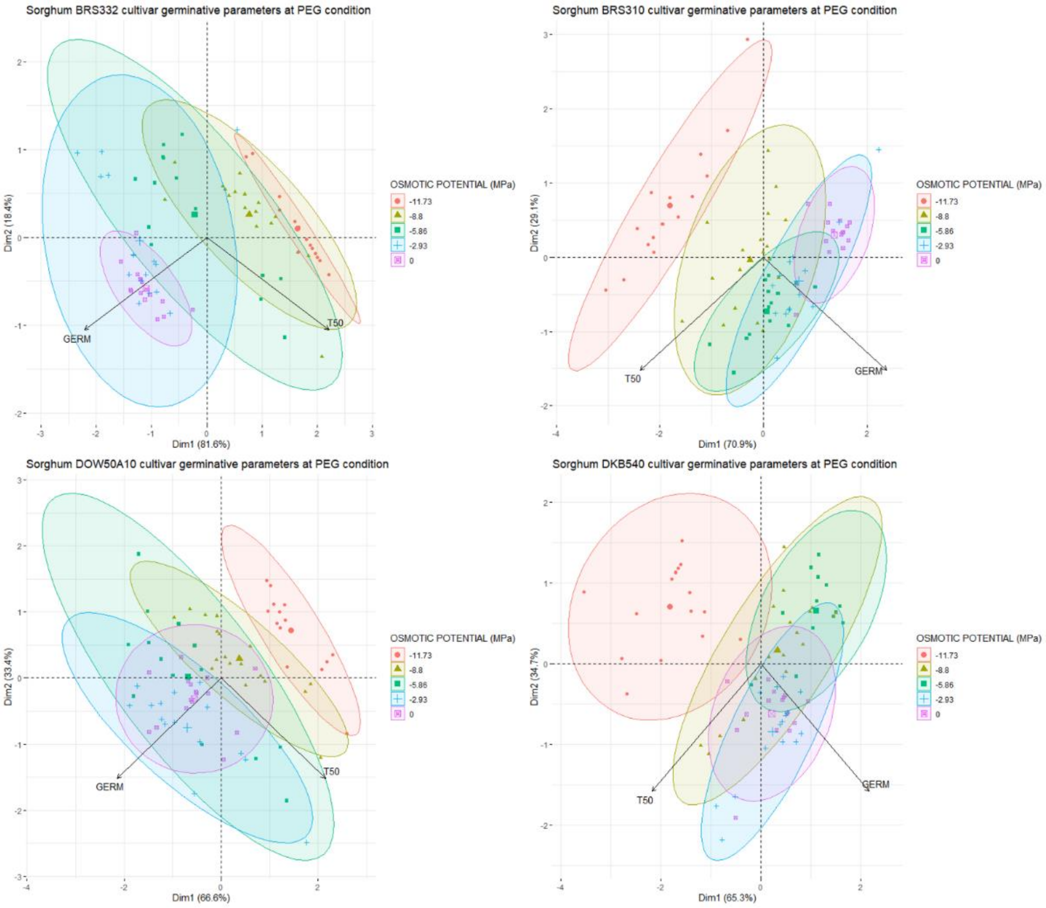
Figure 1.9- Principal component analysis of the effects of water solutions contend 60, 120, 180, and 240 mM NaCl on four sorghum cultivars (BRS-310, BRS-332, DKB 450 and DOW 50A10) primed with 100µM of AsA, ABA, hydroprimed and non-primed.



Source: Ferreira (2022)

There was a negative correlation between the variables for the cultivars BRS-332 and DOW under the condition of osmotic stress simulated by PEG-6000 (Fig. 1.10). It is possible to observe a gradual distancing of axis 1 (81.6%) as the water potential increased. At the lowest potential, there was a greater proximity to the variable T50, that was negatively affected (longer time to reach 50% germinated seeds). Similarly, the cv. DOW50A10 showed a negative correlation, however, even at the highest potentials, it was possible to observe negative changes in the G. The cv. BRS-310 exhibited effects of the higher potentials due to a greater proximity to the axis of the T50 variable, although until the potential of -8.8 MPa there was still a considerably higher germination. DKB showed high germination percentage at potentials closer to -8.8 MPa, being negatively affected only at the highest potential.

Figure 1.10- Principal component analysis four sorghum cultivars (BRS-310, BRS-332, DKB 450 and DOW 50A10) subjected PEG-6000 simulated osmotic potentials effects (-2.93, -5.86, -8.8, and -11.73)



Source: Ferreira (2022)

4. DISCUSSION

Although the known tolerance of sorghum plants to drought, in this work the seeds were negatively affected by salinity and osmotic conditions, significantly reducing seed germination in distinct levels regarding the cultivar evaluated. Even tolerant cultivars, i.e. BRS310, showed sensibility to salt and osmotic stressful conditions. On the other hand, BRS332 demonstrated relative sensitivity and more tolerant than DOW50A10 cultivar. The dissociation of ions in the aqueous solution of soil can cause both osmotic stress and ionic stress due to ions imbalance and its interferences on water potential (Ceritoglu *et al*., 2020). Moreover, regions that face problems due to soil salinity usually also face drought stress conditions, considerably affecting crop establishment, mainly regarding the early stages of plant life cycle (Ibrahim et al., 2016). Therefore, the use of priming technique with known conditioners could provide better acclimation and tolerance for sorghum seeds cultivated in saline soils.

Ascorbic acid is one of the most important antioxidants found in plants, avoiding damage losses, increasing germination timing (Kumar et al., 2013). In this work, the application of priming with AsA was able to promote benefits to seed germination. Under the different stress conditions, the priming was able to provide an increase in germination, especially at higher potentials and at higher doses of NaCl. Similarly, the priming with ABA was also efficient in promoting benefits in germination. Similar effects were observed in experiments conducted by Bahrabadi et al. (2022) when corn seedlings were primed with ABA and other hormones, and by Shah et al. (2019) when wheat seeds were subjected to doses of AsA. Even the hydropriming, as showed here, was able to standardize seed germination under stressful conditions at the lower concentrations in the tolerant and one sensitive cultivar. It indicates that the priming technique *per se* is able to improve germinability and tolerance against osmotic stress in sorghum seeds.

In this work, we verified that salinity and osmotic stress caused changes in the germination of all cultivars, even those tolerant to water stress. BRS-310 and DKB 540 had reductions in germination, however, the most affected cultivars were those susceptible to drought (BRS-332 and DOW 50A10), which affected germination even at lower NaCl doses and higher osmotic potentials. Similar to these results, Dehnavi et al. (2020) observed a decrease in germination of sorghum cultivars according to increasing NaCl concentrations. However, under the lower NaCl doses, an increase in germination can be observed, Nimir at al. (2017) obtained interesting results with sorghum seeds showing an improvement in germination subjected to doses of 50mM, thus a type of priming can be associated with these low doses, promoting a better germination in a short period.

The germination speed index showed changes especially at higher doses (180 and 240mM). At these doses, even when primed, a decrease in this index could be observed, indicating that germination was compromised, especially in cultivars susceptible to water deficit (BRS-332 and DOW 50A10) exposed to drought simulated by PEG-6000. The cultivar DKB performed similar in both conditions (salinity and osmotic stress), however, BRS-310, also tolerant to water stress, showed differences between the two conditions. We can assume, therefore, that the lower osmotic potential caused a delay in germination. It is worth noting that increasing the salt dose can lead to an increase in the time it takes for germination to occur, or even dormancy (Chen *et al*, 2020; Rajabi Dehnavi *et al*., 2020).

The average germination time (T50) reflects the germination speed index; with this parameter, it is possible to stipulate the effect of the two conditions on seed germination. It was observed that there was an increase in the T50 of seeds from the susceptible cultivars BRS-332 and DOW 50A10 also from the tolerant BRS-310. As T50 increases, we can start to see some delays in germination as NaCl doses increase, these changes are observed by several authors in experiments with other plant species (Önal Aşçı and Üney, 2016; Chen *et al*.,2020). The mean germination time (T50) also reflects the speed of seed germination (Bijanzadeh and Egan, 2018). However, in this study, it was verified that the time to reach 50% of seed germination (T50) was extended due to increasing salt content for all sorghum cultivars. In other studies, T50 was also significantly affected it was reported that salt dosages extended germination time and there were differences between cultivars in MGT due to this fact, we can infer that the higher salt concentrations were responsible for influencing lower germination of these materials by significantly delaying germination.

Interestingly, in this study, it was observed that the effects of PEG6000 were more effective to separate the germination parameters of all cultivars, mainly those susceptible. In fact, the cultivars studied here are known to be tolerant or sensible to drought and less information is available of them regarding salinity. This way, probably the lower osmotic potential was more effective on reducing germination percentage and velocity in sorghum seeds studied here. Although salinity and drought stress are physiologically related and the tolerance mechanisms overlap, some aspect of seed physiology and metabolism may differ when the plant experiments single saline and water stresses simultaneously (Sucre and Suárez 2011). In this work we clearly verified the differences on germinability and velocity index of four sorghum cultivars in salt and osmotic conditions. Both tolerant cultivars showed higher improvement in germination parameters when the seeds were primed, independent of the kind of conditioner. For both susceptible cultivars, the priming effects were not able to reduce the restrictions imposed by the treatments, decreasing germinability. However, for either tolerant or susceptible cultivars it was possible to verify that were differences on the levels of tolerance or sensibility.

The cultivar BRS332, even characterized as sensitive, demonstrate huge capacity to germinate in concentrations below 180mM of NaCl regardless of the kind of priming treatment. It suggests that the use of seed priming in this cultivar can be an interesting option for moderate salt soils. On the other hand, almost no differences were verified on germination of the cultivar DBK540 independent of the concentration of salt or water potential. This cultivar, definitely, presents extreme tolerance to salinity and drought. This way, the decreases in germination remains that different damages may be responsible for this impairment, as observed in some studies, including damage to membranes and other structures, as well as damage at molecular levels (Zhang *et al*., 2020; Huang., 2018). Therefore, further experiments comparing mechanisms and strategies of both tolerant and susceptible cultivars are invited.

Evaluating germination parameters in the laboratory is a sure way to ensure that environmental conditions will not be limiting factors for germination and consequent establishment of the crop. All the parameters evaluated in this work are efficient indicators to demonstrate a predictability of what to expect for the seeds in the field, and thus, to adopt measures that can circumvent the deleterious effects that may occur. Therefore, breeding programs as tools for phenotyping could opportunely, incorporate the prediction results with seeds in the laboratory conditions.

5. CONCLUSIONS

Even under high salinity and high osmotic potential conditions, we could observe a beneficial effect of the priming application on the seeds, even though there was a delay in germination, the priming was efficient in promoting germination at higher salt doses and lower osmotic potentials. In general, the priming was efficient and brought benefits to the germination, mainly for the susceptible to water deficit cultivars, which showed a performance similar of the tolerant cultivars. It is still necessary to understand the metabolic effects provided by priming with ascorbic acid and ABA, since the results coming from their application could be used as guidelines for the development of cultivars that adapt to the various realities faced by breeders of this species. Studies that also point out if these benefits are desirable to be performed in seedlings and plants are necessary in order to fill an existing gap in the works carried out using the priming technique.

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CHAPTER II

PRIMING TECHNIQUES: IMPROVING GERMINATION THROUGH DIFFERENT MECHANISMS

ABSTRACT

Affecting crop production, salinity and osmotic stress can promote various impacts on plants. These conditions directly affect seed metabolism and fitness, causing lower germination through the imbalance of osmotic potentials. In the scenario where global changes are increasing, irregular precipitation leads to higher ion accumulation in the soil, as well as the impact of drought technique that can promote better germination and seedling establishment are the focus of the seed scientist, as these processes are highly affected by these conditions. Seed priming appears as a promising technique with lower costs and high efficiency. The use of different molecules in this procedure is investigated in promoting a more efficient antioxidant enzyme metabolism and the accumulation of osmoprotectants. In our work, we observed that the application of the priming technique with ascorbic acid (AsA) and abscisic acid (ABA) increased antioxidant metabolism and proline accumulation in sorghum plants, as well as carbohydrates, promoting an adjustment of seed homeostasis. It is possible to observe in our work that there was a symmetry in the germination percentage (%G) of the cultivar DKB 540, as an increase in the concentration of NaCl and also a decrease in the osmotic potential, however, even under the application of priming this symmetry could not be observed in the cultivar BRS-332. The concentration of MDA and H_2O_2 was lower in the cultivar BRS-332, either under osmotic stress or exposed to higher doses of NaCl, causing it to behave in the same way as the cultivar DKB 540 when submitted to osmotic stress. The enzymes of antioxidant metabolism showed no statistical difference, being the antioxidant potential of amino acids such as proline possibly responsible for maintaining the redox and osmoprotective functions of the seeds submitted to stressful conditions.

Key words: oxidative stress; climate change; hormopriming; abiotic stress, prolin**e.**

1. INTRODUCTION

Improving seed germination and quality is a constant pursuit in agronomy in the present days. Several environmental conditions significantly affect crop establishment, and controlling their effects is a way to achieve better yields in the field and promote food security (Ashraf *et al.*, 2018). Among these limiting factors, the imposition of the osmotic and salinity stress stand out, since these conditions are closely linked and impose restrictions on the germination process (Ibrahim, 2013; Molazem et al., 2015, Corwin, 2021).

Osmotic and saline stress are considered one of the world's most detrimental abiotic stresses and impacts agricultural yields significantly (Nimir et al., 2020). The insecure global climate with irregular rainfall patterns are the major causes of the recurrent onset of water stress worldwide (Moussa and Hassan, 2016). Salt and water stress are both related because they reduce the osmotic potential of the system and compromise water uptake by the seeds (Çakmakçi et al., 2019). The effects of salt stress occur firstly by affecting physiological processes, such as water uptake due to the low water potential of the environment, as well as by the increase of Na^+ and Cl^- ions that are cytotoxic and inhibit the germination process (Yucel and Heybet, 2016; Goharrizi et al., 2020). Therefore, salinity can lead to seed losses due to impairment of generation of reactive oxygen species (ROS) that consequently increase oxidative damage to lipids, proteins, and nucleic acids (Rajabi Dehnavi et al. 2020; Bailly 2019).

The costs and the time expended to develop tolerant cultivars sometimes are not able to overcome the increase in these conditions, at this point, some techniques show more affordable in overcome the restrictions imposed to seeds and plants (Shakeri et al., 2017; Çakmakçı and Dallar, 2019). The seed priming has been presented as an alternative aiming to provide better conditions for germination and overcoming unfavorable conditions, increasing seed tolerance (Ashraf et al., 2018; Aziz et al., 2021).

Seed priming is one of the oldest techniques to obtain better results in germination synchronizing and seedling vigor (Ashraf et al., 2018). Physiologically, it consists in a metabolic preparation of the seeds to unfavorable conditions through controlled imbibition that culminates in faster and uniform germination. The priming consequently improves growth and development of seedlings both under normal and stress conditions (Dey et al. 2014; Nayban et al. 2017). Seed priming induces antioxidant activity and storage protein solubilization and minimizes lipid peroxidation, preserving the membrane functions (Huang et al., 2016). Moreover, this technique is being used for increasing cross-tolerance in seedlings and plants cultivated under unfavorable conditions (Lutts 2016). By way of conceptualization, in this work, the priming technique will be considerate as seed imbibition until phase II of germination, drying and rehydration of the seeds (Lutts 2016; Rhamam et al., 2018).

Usually, the priming includes imbibition with solutions, like phytohormones and signaling molecules. Abscisic acid is one of phytoregulators used for priming technique (Safari et al., 2018). In addition to playing an important role in regulating germination, ABA also presents itself as responsible for promoting tolerance to osmotic stress and seed desiccation reducing their negative effects (Fujita et al., 2013). Another important molecule for being used in priming, that plays a crucial role in plant metabolism, is ascorbic acid (AsA). This molecule is an important antioxidant and enzyme cofactor, and shows regulatory functions in stress situations, promoting tolerance to unfavorable conditions, such as osmotic stress and high salinity (Kumar et al., 2016; Dinler et al., 2014). The exogenous application of this molecule promotes tolerance mainly by enhancing the activity of some enzymes and by minimizing the formation of hydrogen peroxide (H_2O_2) (Naz et al., 2016; Akram et al., 2017; Akram et al., 2019). This way, both conditioners are able to induct tolerance against salinity and osmotic stresses during seed germination.

Standing as one of the world's top five cereal crops, sorghum shows increasing area under cultivation in arid, semi-arid tropical, subtropical, and temperate regions. It is an important source of food, animal feed, and it is a promising bioenergy crop (Marsalis et al., 2010; Rajabi Dehnavi et al., 2020). Sorghum is a moderately salt and drought tolerant crop and can be cultivated in different areas using tolerant varieties. The chosen of the variety to be used is important because the first stages of plant development could be extremely affected even in drought-tolerant varieties (see chapter 1). Due to the sensibility of seeds and seedlings to stressful conditions, it is also important to employ techniques that increases their tolerances. This way, tolerant cultivars and techniques such as priming are employed in order to provide conditions for achieving maximum crop yields (Nimir et al., 2020; Al-Naqeeb et al., 2018, Nimir et al 2015).

Therefore, here we hypothesize that primed-seeds of contrasting-tolerant varieties of sorghum induce different mechanisms to counteract the negative effects of salinity and osmotic conditions. We used hydropriming and priming with ABA and AsA in sorghum seeds cultivated in in osmotic and salinity conditions to evaluate germination and biochemical compounds. This way, the aim of this work was understand how priming types can improve germination, understanding which physiological responses are triggered in different cultivars under saline and osmotic treatments. This work brings important information to obtain a better efficiency of plants and how metabolic responses here studied can guide the use of this technique for sorghum plantations.

2. MATERIAL AND METHODS

2.1. Plant material

All varieties used in the experiment were provided by Embrapa Maize and Sorghum, with the cultivar BRS-332 developed by the breeding program of the institution. The cultivar DKB 540 is a commercial variety developed by Dekalb. The materials were chosen following the criteria of evaluating a drought tolerant cultivar (DKB 540) and a drought susceptible cultivar (BRS-332).

2.1.1. Experimental conditions

The experiment was conducted in the Laboratory of Plant Growth and Development (LCDP) of the Federal University of Lavras (Lavras-MG, Brazil). The experiment was designed in a 3x4 factorial scheme (3 doses of NaCl or their respective osmotic potential versus 4 priming conditions). An individual experiment was conducted for each cultivar, in each experimental condition. The seeds were exposed to priming with the solutions of ABA or AsA or hydropriming for the stipulated period through the imbibition curve (until half of phase II of the imbibition curve was reached). The seeds of control were not primed. After this period, seeds were placed in petri dishes, contain the NaCl solutions: 0, 180 and 240mM, or the respective osmotic potentials simulated by PEG-6000, control (using only deionized water), -8.8, and -11.73 MPa Then, the Petri dishes were placed in a germination chamber, at 25° C with a phopriod of 12/12h at 40µmol photons m^{-2} s^{-1}.

2.1.2. Priming procedure

The priming doses were previously determined according to pre-tests and based on doses found in the literature. The seeds were exposed to the solution containing phytoregulator ABA and AsA for a period of 8 hours, in order to reach the half of phase II, period established according to the tests performed to estimate the imbibition curve. The doses of hormone and ascorbic acid were set at 100 µM, according to the results obtained in the pre-test. In order to guarantee that the materials were homogeneous and contained the same water content, the seeds were dried in a forced circulation oven for 12 hours at 30°C, until achieve the initial water content.

2.1.3. Germination test

The germination of the seeds was monitored for 8 hours. The criteria for considered germination was the protrusion of 2mm of radicle. This parameter was established to ensure that no seedlings were collected for biochemical analysis.

2.1.4. Sampling

At 48 hours the seeds were taken and washed with deionized water to remove residues of NaCl or PEG-6000 solutions. After washing, the seeds were wrapped in aluminum foil, identified and stored in liquid nitrogen and then stored in an ultra-freezer (-80°C). For the biochemical analyses, a pool of seeds (n=10) was made to compose one replicate. The seed pool was macerated with 1% PVPP (polyvinylpyrrolidone) and liquid nitrogen for extraction preparation.

2.1.5. Antioxidant enzymatic assays

For the enzyme extraction (catalase - CAT, superoxide dismutase - SOD and ascorbate peroxidase - APX), 100 mg of macerated seeds were homogenized with 1 mL of phosphate buffer (100 mM pH 7.8) containing 100 mM ethylenediaminetetraacetic acid (EDTA), 1 mM ascorbic acid and 5% polyvinylpyrrolidone (PVP) (Gomes et al., 2014). Protein dosage was carried out by Bradford method (Bradford, 1976).

Catalase (CAT): the reaction medium for CAT was composed of 67 mM potassium phosphate buffer (pH 7), 10 mM H_2O_2 and enzyme extract. H2O2 consumption was measured at 240 nm ($\mathcal{E}$ = 36 M^{-1} cm^{-1}) as described by Anderson et al (1995).

Superoxide dismutase (SOD): SOD activity was measured in reaction medium containing 50 mM phosphate buffer (pH 7.8), 13 mM L-methionine, 0.1 mM EDTA, 0.002 mM riboflavin and 0.075 mM NBT as described by Giannopolitis and Ries (1977). The reaction was conducted under fluorescent light (15 W) for 10 minutes and in the dark at room temperature. The formation of formazan blue, derived from the reduction of NBT, was measured spectrophotometrically at 575 nm. One unit of SOD was defined as the amount of enzyme required to inhibit NBT reduction by 50%.

Ascorbate peroxidase (APX): APX activity was measured in medium composed of 50 mM phosphate buffer (pH 7), 0.5 mM ascorbic acid, 2 mM H_2O_2, and appropriate amount of enzyme extract. Ascorbate oxidation was monitored at 290 nm ($\mathcal{E} = 2.8\ mM^{-1}cm^{-1}$) as described by Nakano and Asada (1981).

2.1.6. Proline content

The method used was the one described by (CARILLO & GIBBON, 2011). Samples with 0.1 g of seeds fresh matter were homogenized with 60% ethanol and centrifuged at 14000g for 5 minutes. In a test tube containing 0.5 ml of the supernatant, 1 ml of added 1 ml of 1% ninhydrin in 60% acetic acid and 20% ethanol. Then the samples were kept 20 min in a water bath at 95º C. After cooling, by immersion in an ice bath, 1 ml of 60% ethanol was added, and the samples were read in a spectrophotometer at 520 nm. The absorbances obtained were compared with the standard curve of proline. The results obtained are expressed in micrograms of proline per $\mu g.mgMS^{-1}$

2.1.7. Total soluble sugars

For the quantification of total soluble sugars (TSS) the anthrone (hydroxyanthrancene) method was used (YEMM and WILLIS, 1954). For this, 40 mg of was weighed and then 1.0 mL of distilled water was added. Subsequently, it was added 20 mL of concentrated sulfuric acid (H_2SO_4). 50uL of crude extract was added and then the anthrone reagent. After the solution was prepared, the tubes were vortexed and placed in a 100ºC water bath for three minutes. Finally, it was read in a spectrophotometer at a wavelength of 620nm.

2.1.8. Reducing sugars

To perform the quantification of reducing sugars, the DNS method was used which contains the following reagents: dinitrosalicylic acid (DNS), sodium hydroxide, double tartrate sodium hydroxide, double sodium tartrate (Rochelle salt), glucose (10 mM). For the realization of analyses, stock solutions were made. To this end, 50 mL of sodium hydroxide 2.5g of DNS and 125 mL of distilled water. The solution was then stirred until its total dissolution.

Subsequently, 75g of Rochelle salt was added and made up in 250 mL with distilled water. Subsequently, the solution was submitted to a water bath at 100ºC for five minutes. After cooling, readings were taken in a spectrophotometer with a wavelength of 540nm.

2.1.9. Quantification of total soluble amino acids

The method described by Yemm & Cocking (1955) was used to quantify the variations in the concentration of total soluble amino acids present in the seeds. After obtaining the extract in a volume of 1 ml, 500 μl 0.2 M sodium citrate buffer, pH = 5.8, 200 μl ninhydrin 5% solution in monomethyl ether of ethylene glycol + 1 ml of 0.0002 M KCN solution. The assay was heated at 100° C in a water bath for 20 minutes, then cooled for 10 minutes and 1 ml of 60% ethyl alcohol was added 60%, followed by spectrophotometer reading at 570nm and the results were expressed in μmol.gMS^{-1}.

2.1.10. Statistical analysis

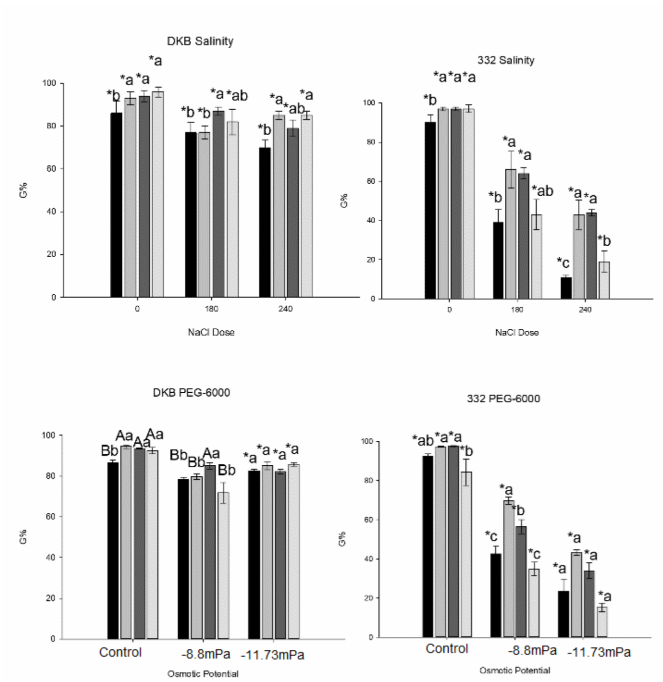
The experiment was carried out in completely randomized blocks in a 3x4 (3 NaCl doses x 4 priming types) in a factorial arrangement, with a collection at 8 hours (Middle of phase II of imbibition curve). Statistical analyzes were performed using the Rbio software (Bhering, 2017). The data were subjected to ANOVA and, when in normal distribution, the Tukey means test at 5% significance was performed.

3. RESULTS

Under salinity and osmotic conditions, the final germination percentage of the sensible cultivar (BRS-332; Fig. 2.1) which seeds were subjected to priming with ASA and hydropriming showed the greater germination under both conditions. It was possible to observe a decrease in the germination percentage following an increase in the salt concentration and in the higher osmotic potentials in this cultivar.

For the drought-tolerant cultivar (DKB 540; Fig. 1), it was possible to observe that even in condition of salinity and osmotic stress, the seeds showed germinability higher than 70%, mainly those AsA and ABA-primed.

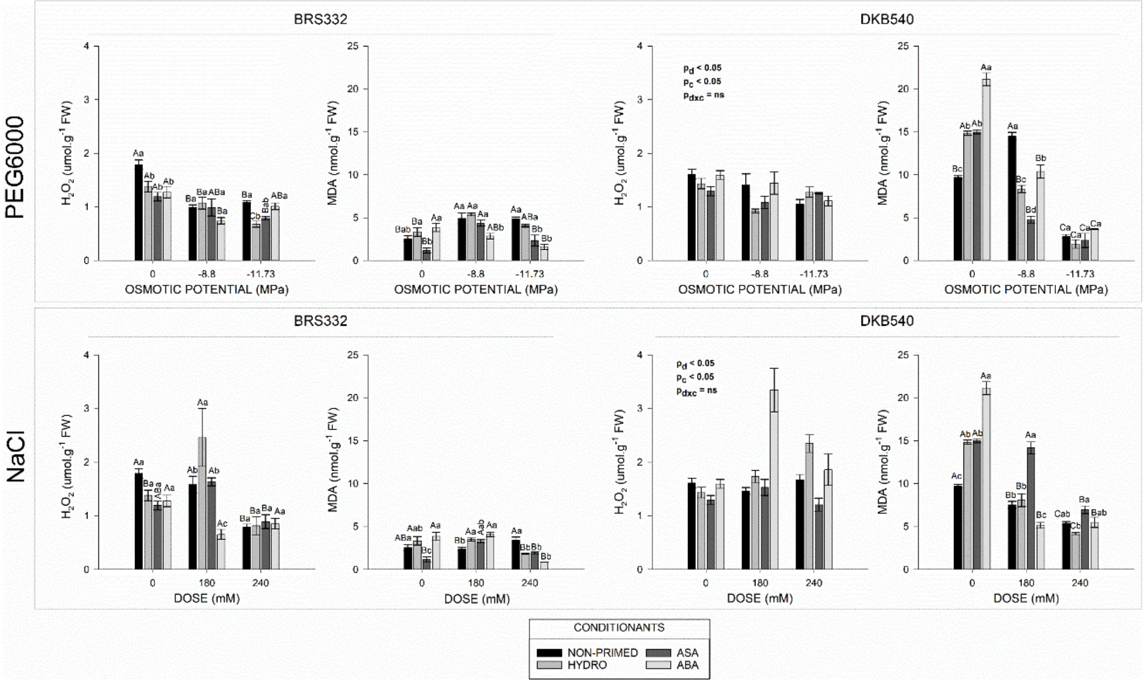
Figure 2.1 Final germination percentage of the cultivar BRS-332 and DKB 540 subjected to PEG-6000 solutions at respective osmotic potentials control (0), -8.8, and -11.73, and to solutions of water with 0, 180, and 240 mM NaCl. Uppercase letters compare doses inside conditioning, lowercase letters compare conditioning inside doses or osmotic potential.



Source: Ferreira (2022)

For the both stressing conditions, it is possible to observe (Fig.2.2 A-D) that the hydrogen peroxide (H_2O_2) concentration did not increase considerably following the salt concentrations or the osmotic potential in both cultivars. The MDA concentration (Fig. 2.2 E-H) in the cultivar BRS-332 under salinity was relatively low however in the cultivar DKB 540 its possible observe a increase in the oxidative stress marker, even, these increase did not affect the germination, indicating that this variety have a great efficiency in circumvent the damage caused by the ROS.

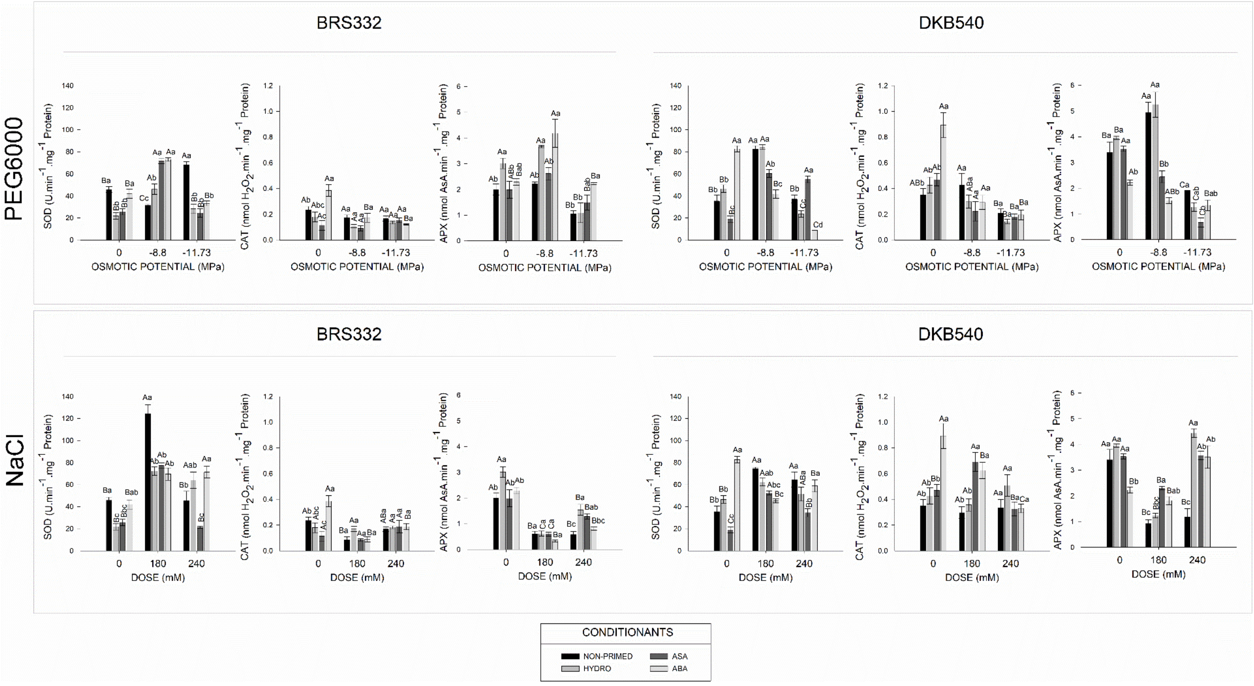
Figure 2.2- Hydrogen peroxide and MDA concentration of cultivars BRS-332 and DKB 540, primed with ascorbic acid (100µM), abscisic acid (100µM), hydro priming and non-primed subjected to salinity (0, 180, and 240 mM NaCl) and osmotic stress control, -8.8, and -11.73, Bars represent means ± standard error. Upper case letters compare the difference between the applied priming, and lower case letters compare the osmotic potentials or NaCl doses (*n*=4). Means followed by the same letters or Ns (non-significant) do not show significant difference by Tukey test at 5% significance level.



Source: Ferreira (2022)

The activity of the antioxidant enzymes (Fig. 2.3 A-N) shows a higher activity of these enzymes in the cultivar DKB-540 comparing to BRS-332. It could be observed mainly regarding to the application of priming with ABA and AsA, especially in conditions of high salinity and osmotic stress for DKB-540. The BRS-332 cultivar showed a considerable increase in enzyme activity under conditions of 180mM NaCl and respective osmotic potential.

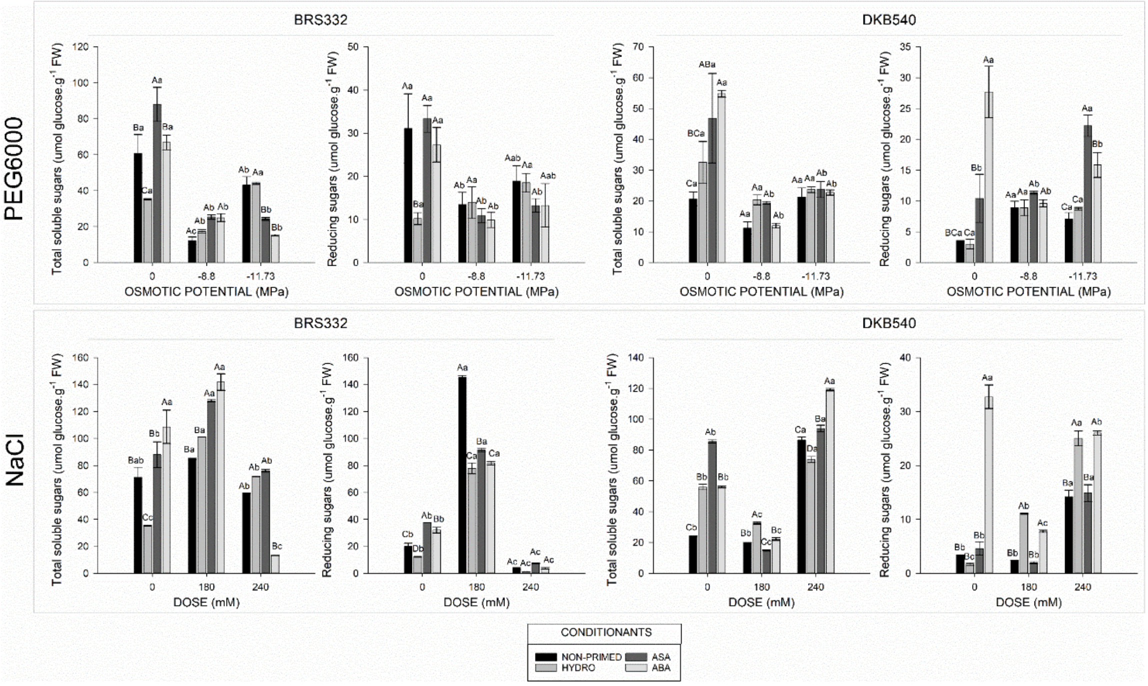
Figure 2.3- Antioxidant system (Superoxide dismutase (SOD), Catalase (CAT) and Ascorbate peroxidase (APX)) activity of cultivars BRS-332 and DKB 540, primed with ascorbic acid (100µM), abscisic acid (100µM), hydro priming and non-primed subjected to salinity (0, 180, and 240 mM NaCl) and osmotic stress control, -8.8, and -11.73,, Bars represent means ± standard error. Upper case letters compare the difference between the applied priming, and lower case letters compare the osmotic potentials or NaCl doses (*n*=4). Means followed by the same letters or Ns (non-significant) do not show significant difference by Tukey test at 5% significance level.



Source: Ferreira (2022)

The concentration of total soluble sugars and non-reducing sugars changed as there was an increase in NaCl dose and a decrease in osmotic potential (Fig. 2.4 A-H). The cultivar BRS-332, drought-sensitive, showed an increase in TSS at doses of 180mM NaCl, while under osmotic stress conditions it is possible to observe an increase in NRS content. The cultivar DKB-540 showed an increase in the content of TSS and NRS when priming with ABA and AsA was applied. The highest contents could be observed in high salinity conditions, both for TSS and NRS, especially for the application of priming with ABA.

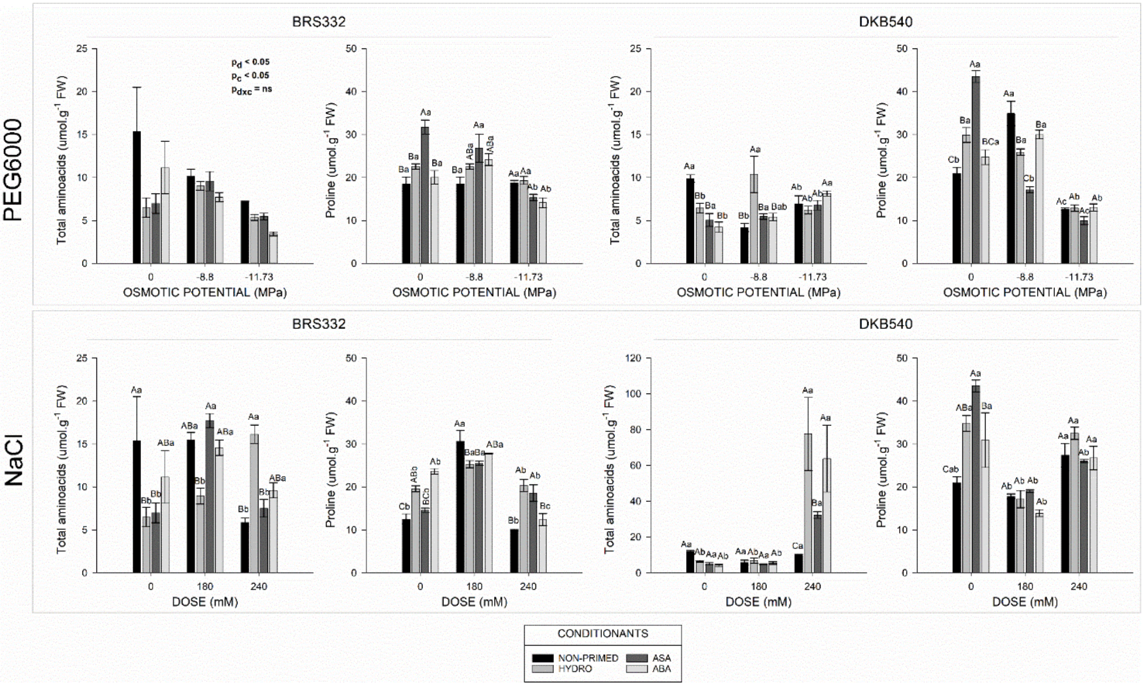
Figure 2.4- Total Soluble sugars and reducing sugars concentration of cultivars BRS-332 and DKB 540, primed with ascorbic acid (100µM), abscisic acid (100µM), hydro priming and non-primed subjected to salinity (0, 180, and 240 mM NaCl) and osmotic stress control, -8.8, and -11.73, Bars represent means ± standard error. Upper case letters compare the difference between the applied priming, and lower case letters compare the osmotic potentials or NaCl doses (*n*=4). Means followed by the same letters or Ns (non-significant) do not show significant difference by Tukey test at 5% significance level.



Source: Ferreira (2022)

The data in Figure 2.5 (A-H) show the results for total amino acid (TAA) and free proline (Pro) concentration on that it is possible to observe that there is an increase in the TAA and Pro contents in the cultivar BRS-332 when submitted to the priming application, this increase occurs both in the control treatment and in the 180mM NaCl dose. However, it can be observed that when submitted to the 240mM and the respective osmotic potential, there is a decrease in this content. The cultivar DKB-540 showed a variation of TAA and Pro content in both stressing conditions. It was possible to observe that there was a lower content of TAA in osmotic stress conditions for the cultivar DKB 540, even under the lowest potentials it is possible to observe this lower content, however there is a considerable increase of TAA in the highest dose of NaCl (240mM), this increase is related to the application of priming with both ABA and AsA.)

Figure 2.5- Free amino acids and proline concentrations of cultivars BRS-332 and DKB 540, primed with ascorbic acid (100µM), abscisic acid (100µM), hydro priming and non-primed subjected to salinity (0, 180, and 240 mM NaCl) and osmotic stress control, -8.8, and -11.73,, Bars represent means ± standard error. Upper case letters compare the difference between the applied priming, and lower case letters compare the osmotic potentials or NaCl doses (*n*=4). Means followed by the same letters or Ns (non-significant) do not show significant difference by Tukey test at 5% significance level.



Source: Ferreira (2022)

4. DISCUSSION

The use of seed priming aims to improve germination physiology and stress tolerance according to the use of different molecules or even water. With the results showed in this work, it was possible to identify differences in the mechanisms accessed by the sensitive and drought-tolerant cultivars regarding the type of the priming applied. The priming technique, although being considered low-cost, has great implications in providing benefits to seeds, as showed here. The use of growth regulators, as ABA well-reported to abiotic stress signaling, and antioxidant molecules, as AsA, widely distributed in plant tissues, as conditioners are widely reported in the literature (Lutts et al., 2016; Liu et al., 2019; Oliveira et al., 2019). However, the mechanisms of how they can increase seed tolerance through priming application against salinity was not reported yet.

There are differences in the mechanisms observed for the varieties, in DKB 540 its possible observe that the increase in salt doses or in the osmotic potential there's no significant impacts on the germination, while BRS-332 suffers with these alterations. Even presenting a decrease in the germination, we couldn't observe cues that leads us to affirm that oxidative damage has occurred, the levels of hydrogen peroxide and MDA have changed under the different concentrations of NaCl and the different osmotic potential, but they have been circumvented in all priming types. In DKB 540 is possible observe a higher activity of CAT in the salinity stress but this enzyme didn't appear with higher activity in osmotic stress however, APX showed an efficient activity in prevents the oxidative damage for this cultivar in both conditions. BRS-332 showed a lower activity of CAT in osmotic and salinity stress, and a lower activity of APX in salinity conditions, but SOD showed a great efficiency in both conditions, and a higher activity of APX when seeds are subjected to different osmotic potential.

The final germination of both cultivars was differently affected. While the seeds of the cultivar BRS-332 decreased considerably the germinability mainly without priming, DKB seeds maintained germination above 70% in all treatments. In BRS 332 seeds primed with water and AsA, the germination capacity was greater than the other treatments in salinity (180 and 240mM) and osmotic (-8.8MPa) conditions. The effects observed promoted by the priming application could promote conditions to avoid the oxidative damage but was not able to promote the increase the germination percentage, it's possible to affirm that these effects were promoted by the molecules adopted, since the AsA in highly referenced as an efficient antioxidant and the changes through the application of hydropriming is correlated to the AsA use. The high capacity to keep germinability under stressful conditions, is desirable for tolerant cultivars, mainly to those crop species that are widely cultivated, including high salinity or drained soils, as sorghum. The higher germinability of DKB540 seeds in unfavorable conditions in germination chamber was previously tested (see chapter 1), and confirmed here. The superiority of grain yield and tolerance to abiotic stresses of DKB540 related to BRS332 was previously reported by Santos et al. (2018) and by Batista et al. (2017). There is important to point out that high germination capacity in distinct abiotic stresses can improve seedling vigor and crop establishment. Therefore, there was investigated the mechanisms that differentiate both cultivars in the conditions of these experiments.

In high salinity or low osmotic potentials, that are limiting conditions to seed germination, it is expected increasing in oxidative stress (Zhao et al. 2021). Under osmotic stress and salt stress, the effect of priming was more efficient in promoting greater protection of the seeds from oxidative stress. The peroxide content observed under osmotic stress conditions was lower than the content observed in seeds exposed to high salinity. The cultivar BRS-332 showed a lower content of MDA in both conditions, however, at the higher dose and lower osmotic potential the efficiency of priming with AsA and ABA was more prominent (Matias et al., 2018; Farooq et al., 2019). Therefore, the oxidative stress it was not observed on both cultivars tested here. The levels of MDA, a marker of oxidative stress (Du and Bramlage, 1992), related to the levels of H2O2, in both cultivars did not indicate oxidative damage. It can be assumed, therefore, that germination reduction in BRS 332 cultivar was not due to membrane impairment or damage. The saline and osmotic treatments could probably have delayed seed germination in this cultivar during the evaluated time, and clearly, the effect of priming with ABA and AsA protected the seeds against damage. For DKB 540 seeds, there was an increase in MDA levels, but the germination percentage was not affected in both osmotic or salinity conditions, independent of priming treatments. It is expected, in this way, an effective antioxidant system acting in these seeds.

As can be observed, the DKB 540 seeds showed increased antioxidant enzymatic activity regarding BRS 332, independent of the condition or priming treatment. However, in DKB 540 seeds, the priming with ABA and AsA increased CAT and APX activities in saline and osmotic conditions. These molecules AsA and ABA) probably allowed the seeds of both cultivars to prevent the oxidative damage by promoting a more efficient metabolism of enzymes of the antioxidant system, above all, in the drought susceptible cultivar (BRS-332). Several studies point to an increase in the activity of antioxidant enzymes regarding priming treatments (Luo et al., 2016; Liu et al., 2019; Li et al., 2020), moreover when AsA and ABA was applied exogenously. The SOD activity in the dose of 180mM of NaCl and the respective osmotic potential had a considerably higher enzyme activity, this indicates that under these conditions when found in the natural environment these cultivars can present a better performance since submitted to priming (Hussain et al., 2019). The cultivar BRS-332 in salinity condition presented lower activities of the enzymes CAT and, however, , there was no increase in the marker of oxidative damage (MDA). Although the presence of a high concentration of NaCl led to an increase in the content of H_2O_2 in the cv. DKB 540 that was ABA-primed, this priming treatment provided a better condition for the extinction of this ROS, which was corroborated by the fact that there was a lower content of MDA. This way, we can assume that priming treatments prevent oxidative damage in sorghum seeds under salinity and osmotic conditions by enhancing antioxidant enzymes, mainly in DKB 540 seeds primed with ABA and AsA.

Abscisic acid and ascorbic acid promoted a higher accumulation of total soluble sugars (TSS) and proline in both cultivars under both stressful conditions, however, the accumulations of these sugar are influenced by the concentrations of salt, at the highest dose (240mM of NaCl) the tolerant one (DKB 540) showed the higher accumulation of TSS while the higher amount of these carbohydrates are limited to the dose of 180mM in the drought susceptible (BRS-332). The application of these two molecules is reported in seeds submitted to limiting conditions of water availability (low osmotic potential), and under high salinity conditions. This greater accumulation is due to the osmotic adjustment; in this sense both TSS and Pro play this role, as well as, with the increase of salinity and also the decrease of osmotic potential enabled this greater accumulation, once the mobilization of reserves for germination is affected in detriment of osmotic protection (Kumar et al., 2017). Regarding the cv. DKB 540, it is important to note that these seeds showed the capacity to maintain the accumulation of proline and sugar even under high salinity or low osmotic potentials. This way, the priming was not definitive on osmotic protection of the seeds from this cultivar probably due to its inherent capacity to produce them. Clearly, the benefits of priming, mainly ABA and AsA, for seeds of BRS 332 cv. were more prominent.

In our study we could verify that although the effects of osmotic stress and high salinity are perceived by the seeds, the application of priming was efficient in alleviating the effects of osmotic stress that is associated with the presence of a lower osmotic potential or even by the presence of toxic ions due to the dissociation of NaCl. The accumulation of solutes can be a tool to be explored by growers through the application of priming, due to the low complexity and also the benefits obtained.

The mechanisms elucidated here in our work bring to light important information on the physiological aspects of the modulation of sugar accumulation and also of compatible osmolytes, understanding the crosstalk between AsA and ABA may generate new perspectives on the activation of these stress tolerance mechanisms by seeds and also seedlings. As it was possible to observe, that the use of different types of priming was beneficial in providing metabolic alterations that allowed the seeds to face adverse conditions. The application of priming with the two molecules (ABA and AsA) brings us evidence that a beneficial effect at higher doses and under lower osmotic potentials is evident (Pastor et al., 2013; Farajollahi and Gholinejad 2014).

5. CONCLUSIONS

In general, it is possible to observe that the effect of priming with ABA and AsA can be beneficial when the seeds encounter detrimental conditions of limited water availability and high salinity. The application of seed priming can lead to the occurrence of cross-tolerance when the seeds are under natural conditions, which is an important advance in the preconditioning research. The use of priming with the hormone ABA and the antioxidant molecule AsA enhancement of antioxidant metabolism, as well as the accumulation of osmoprotectant molecules can ensure cellular homeostasis leading to efficient germination. It is possible to observe that there is a tendency that the application of priming, regardless of the type adopted, promotes benefits especially in the drought susceptible species (BRS-332), since the parameters analyzed here tend to be similar. The tolerant species (DKB-540) showed significant increase in osmoprotectants, indicating that these molecules are important in promoting drought tolerance and should be better studied in other experiments, especially the maintenance of the effects observed in plants. Thus, from the results showed here, it is interesting to understand the effects of priming in seedlings and plants, since after the success of germination, the imposition of various stresses can compromise the establishment of them in arid conditions where the sorghum is cultivated.

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CHAPTER III

THE MEMORY REMAINS: HOW PRIMING PROMOTE SORGHUM SEEDS GERMINATION UNDER OSMOTIC AND SALINITY CONDITION?

ABSTRACT

Metabolic alterations resulting from salt stress have severe impacts on plants. The main changes that can be observed are in the carbon metabolism of plants. The presence of high concentrations of salt in the soil leads to physiological alterations that compromise processes related to the use of resources by plants. Salinity affects the uptake of water, since there are alterations in the osmotic potential, and there are also compromises due to alterations in stomatal conductance that will affect the carboxylation of C. These alterations affect not only the energetic metabolism, but also the accumulation of biomass. The application of priming is an alternative to circumvent these conditions, one of the main positive points of the technique is the possibility of promoting the synthesis of carbohydrates and amino acids such as proline that are able to provide an osmotic adjustment bringing benefits to the plant. In this work, we performed photosynthetic metabolism analysis, as well as chlorophyll fluorescence, gas exchange, and biochemical analysis in plants from seeds that were primed and submitted to salt stress. Our hypothesis is that the application of priming is able to promote benefits to plants submitted to salt stress, due to improvements in osmotic adjustment. The results obtained allowed us to conclude that the application of priming with ASA, ABA and hydropriming were able to circumvent the deleterious effects of salinity, since it was possible to observe that there were no significant compromises in the parameters obtained through the analysis of chlorophyll fluorescence a. Furthermore, the application of priming was able to promote an osmotic adjustment, indicating that the proline content was efficient in guaranteeing that the stomatal conductance was maintained, not affecting the photosynthetic processes.

Key words: oxidative stress; climate change; hormopriming; abiotic stress, prolin**e.**

1. INTRODUCTION

Soil salinity is a condition imposed by the increased concentration of ions that significantly impact agricultural production by limiting plant germination, growth, and development. It is prone to occur especially in areas where there is constant water restriction due to low rainfall, such as arid and semi-arid regions (Ali et al., 2021; Ibrahim et al., 2019; Nimir et al., 2015). It is estimated that approximately 6% of the world's arable areas will suffer salinity problems in the next 25 years. Associated with climate change, 50% of agricultural areas will suffer from an increase in salinity by the middle of the 21st century (Machado and Serralheiro, 2017; Rodríguez et al., 2014). In this way, salinity could be considered one of the most sources of losses in agriculture around the world.

During plant growth and development, several phases are formally affected by high salinity, mainly due to toxicity by the dissociation of ions such as Na+ and Cl-, compromising the osmotic balance and causing a lower nutrient uptake (Farhangi and Ghas- semi, 2018). Crop yield is severely affected by salinity since seedling establishment, one of the most sensible steps of plant development due to the high sensitivity of water loss and desiccation. It is also reported a decrease in photosynthetic metabolism occurs under salinity conditions (Isayenkov and Maathuis 2019). There are different conditions that affect the control mechanisms of growth and biomass accumulation under environmental stress conditions, especially by high salinity (Fatichi et al., 2014). Among these conditions the ones that stand out most are the carbon balance between photosynthesis and photorespiration (Escalona et al., 2003), temperature and the transpiration flux through the leaves (Parent, et al., 2009; Parent, et al., 2010; Pantin, et al., 2011; Muller, et al., 2011; Pantin, et al., 2012).

One of the tolerance mechanisms accessed by plants in high salinity is the fluorescence of chlorophyll a, that consists in the dispersion of thermal energy, through the re-emission of the light photon into the environment (Strasser, 2004). In stressed plants, one way to quantify how compromised the photosynthetic apparatus is to measure the emission flux of chlorophyll a fluorescence. The decrease in the efficiency of photosystem II (FSII), is expressed in the drop in variable fluorescence (Fv) and maximum fluorescence (Fm) (Strasser, 2010).

Through chlorophyll a analysis it is possible to obtain detailed information about the energy transfer in photosynthesis (Maxwell and Johnson 2000, Kalaji et al. 2017). Such mechanisms aim to protect the FSII, more specifically the reaction center of this apparatus, since it is more susceptible to damage caused by various forms of abiotic stress than the photosystem I (FSI) (Vass et al., 2009). Due to this particularity, the damage caused to photosystem II (FSII), requires repairs, mainly in the D1 protein (Foyer et al., 2011; Foyer et al., 2012). The salinity stress becomes a risk for the plant, because as there is a reduction in the pool of $NADP^+$, the FSI electron receptor, there will be a detour of electrons that would be used in the synthesis of CO_2. The impairment of electrons transfer may cause the reduction reaction of intracellular O_2 leading to a higher probability of forming reactive oxygen species, which will cause lipid peroxidation in cells (Lawlor, 1995; Azevedo Neto et al., 2006).

To get around the problems caused by high salinity, strategies and techniques are necessary to mitigate the deleterious effects that reduce plant fitness. The seed priming emerges as a low-cost alternative that is being used as a mechanism of cross-tolerance transference from seeds to plants (Lutts, 2017; Ibrahim, 2016). The priming consists in soaking the seeds in water or solutions in a controlled manner, for a certain period of time for reactivation of metabolism without the conclusion of germination. Then, the seeds are dried being stored and used later. The use of priming in seeds could be, therefore, used for inducing tolerance in crops cultivated in saline soil. The use of phytoregulators, as abscisic acid (ABA), and antioxidant molecules, as ascorbic acid (AsA) in priming solutions emerges as an alternative to induce salinity-tolerance. ABA and AsA are related to signaling pathways of drought-tolerance and increasing antioxidant system (Miceli et al., 2020).

Among cereals, sorghum ranks fifth as the world's most widely cultivated crop, especially in arid and semi-arid regions, as well as tropical and temperate regions. This crop is widely used as a raw material for the production of beverages and for energy production, and is widely used as a food and feed crop feeding more than 500 million people (Pennisi 2009). Although its cultivation is widespread in arid and semi-arid regions, where the occurrence of saline and water stress occurs with a higher incidence, this crop can be classified as tolerant to moderate to abiotic stress. However, even the tolerant crops or cultivars are susceptible to decrease the fitness in high salinity. This way, the use of the techniques that improve salt tolerance is urgent, and the use of seed priming can be a useful method for this purpose.

Therefore, we hypothesize in this work that the application of seed priming with AsA, ABA an hydropriming could promote tolerance to salinity stress through improvements in carbohydrates and proline metabolism in seedling and plants of sorghum exposed to salinity. The aim of this work was to understand if the seedling and plants of sorghum obtained from seeds that have passed for priming are more able to tolerate the effects of salinity stress at initial growth stage and during the pre-anthesis stages, since theses phenological stages are more prone to suffers the effects of salinity stress. To evaluated this, two contrasting varieties of sorghum were used, one tolerant to drought (DKB 540) and other susceptible to drought (BRS-332).

2. MATERIAL AND METHODS

2.1. Plant material and Experimental conditions

Seeds of sorghum (*Sorghum bicolor* (L.) Moench) from Embrapa Milho e Sorgo, Brazil, were used. The experiment was conducted in a greenhouse in the campus of Universidade Federal de Lavras (UFLA), Lavras - MG, Brazil (21º17'33" S latitude, 45º10'41" W longitude and 904 m altitude). Ten seeds of two sorghum cultivars (BRS-332, drought-sensitive; and DKB 540, drought-tolerant) submitted to priming with AsA (100uM) and ABA 100uM), as well as hydropriming, and non-primed seeds (control) were used for the experiments. Prior to the seeding, a solution containing 180 or 240 mM NaCl, or water (control) was applied in each of the pots for induction the salinity treatment. There were used 7L pots containing substrate composed of red latosol mixed in a 2:1 sand ratio, being a ratio of 2 of red latosoil to 1 of sand. The seeds were sowed in these pots and the experiment was conducted for 65 days keeping the pots were at 60% of field capacity.

2.1.1. Sampling

During the experiments, the collection of material was performed at 4 days after germination (seedlings) and at 65 days, when the experiment was finished and samples were collected for root and aerial part analysis, as well as aerial and root length measurements.

2.1.2. Evaluation of photosynthetic responses

Gas exchange measurements were performed during the morning (between 07:00 h and 12:00 h) on the second fully expanded leaf of each plant using an LI-6400xt infrared gas analyzer (Li-Cor Inc., Lincoln, NE, USA). Light (1500 μmol m^{-2} s^{-1}) was provided by using the LED light source in the leaf chamber fluorometer (6400-40, Li-Cor Inc.), with an area of 6 cm2. Measurements were performed using a CO_2 control system (6400-01, Li-Cor Inc.), at 400 μmol mol^{-1} CO_2, with an average leaf temperature and air humidity of 26°C and 45%, respectively. The following gas exchange variables were evaluated: net photosynthetic rate (A, μmol m^{-2} s^{-1}), stomatal conductance (gs, mol m^{-2} s^{-1}), transpiration (Tr, mmol m^{-2} s^{-1}), and intercellular CO2 to atmosphere concentration ratio (Ci/Ca).

2.1.3. Chlorophyll *a* fluorescence

The OJIP transients were measured on the same leaves used for gas exchange measurements using a FluorPen FP100 (Photon Systems Instruments, Brno, Czech Republic). The OJIP transient was determined on dark acclimated leaves for at least 30 min (two measurements per plant).

2.1.4. Photosynthetic Pigments

The total chlorophyll index was determined using the portable chlorophiLOG meter (CFL1030, Falker, Porto Alegre, RS, Brazil). Measurements were performed two times on the middle part of the second fully expanded leaf, and the average of the two measurements was calculated as one replicate.

2.1.5. Lipid peroxidation and hydrogen peroxide concentration measurements

Malondialdehyde (MDA) concentration was determined to assess the level of lipid peroxidation in fully expanded leaves following the protocol of Hodges et al. (1999). Material from the same leaf was used to determine H_2O_2 concentration according to Velikova et al. (2000).

2.1.6. Total soluble sugars

For the quantification of total soluble sugars (TSS) the anthrone (hydroxyanthrancene) method was used (YEMM and WILLIS, 1954). For this, 40 mg of was weighed and then 1.0 mL of distilled water was added. Subsequently, it was added 20 mL of concentrated sulfuric acid (H2SO4). 50uL of crude extract was added and then the anthrone reagent. After the solution was prepared, the tubes were vortexed and placed in a 100ºC water bath for three minutes. Finally, it was read in a spectrophotometer at a wavelength of 620nm.

2.1.7. Reducing sugars

To perform the quantification of reducing sugars, the DNS method was used which contains the following reagents: dinitrosalicylic acid (DNS), sodium hydroxide, double tartrate sodium hydroxide, double sodium tartrate (Rochelle salt), glucose (10 mM). For the realization of analyses, stock solutions were made. To this end, 50 mL of sodium hydroxide 2.5g of DNS and 125 mL of distilled water. The solution was then stirred until its total dissolution. Subsequently, 75g of Rochelle salt was added and made up in 250 mL with distilled water. Subsequently, the solution was submitted to a water bath at 100ºC for five minutes. After cooling, readings were taken in a spectrophotometer with a wavelength of 540nm.

2.1.8. Proline quantification

The method used was the one described by (CARILLO & GIBBON, 2011). Samples with 0.1 g of seeds fresh matter were homogenized with 60% ethanol and centrifuged at 14000g for 5 minutes. In a test tube containing 0.5 ml of the supernatant, 1 ml of added 1 ml of 1% ninhydrin in 60% acetic acid and 20% ethanol. Then the samples were kept 20 min in a water bath at 95º C. After cooling, by immersion in an ice bath, 1 ml of 60% ethanol was added, and the samples were read in a spectrophotometer at 520 nm. The absorbances obtained were compared with the standard curve of proline. The results obtained are expressed in micrograms of proline per µg.mgMS-1.

2.1.9. Statistical analysis

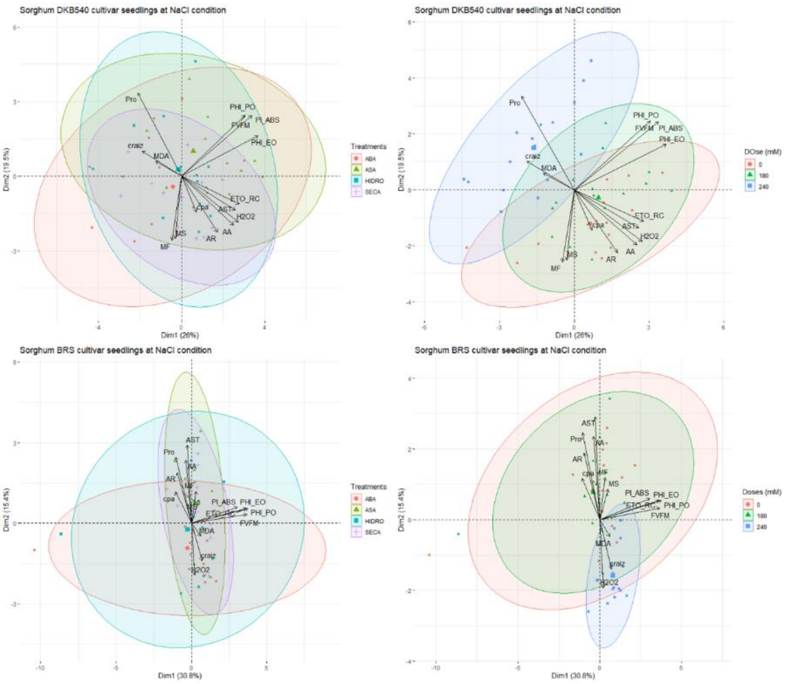
The experiment was carried out in blocks in a 3x4 factorial arrangement, statistical analyzes were performed using the Rbio software (Bhering, 2017). The data were subjected to ANOVA and, when in normal distribution, the Tukey means test at 5% significance was performed. The Principal Components Analysis was performed using the software R (stats and factoextra packages). In the PCA the effects of priming and doses were evaluated for the following parameters: Proline content (Pro), total amino acid content (AA) total soluble sugars (TSA) and reducing sugars (RA) content, biochemical parameters as well as the parameters of photochemical metabolism primary quantum yield of photosystem II (Fv/Fm), electron transport quantum yield of QA- to intersystem electron acceptors (Phi_E0), primary photochemical maximum quantum yield at t = 0 (Phi_P0), reoxidation of QA- via electron transport in an active reaction center (ETo/RC) and electron absorption performance index (PI_abs). The PCA analyses for plants at 65 days after germination (65DAG) in addition to the parameters analyzed in seedlings were added parameters of photosynthetic metabolism, photosynthesis (Photo), stomatal conductance (Cond), transpiration (TR) and internal carbon content (CI), as well as chlorophyll a and b contents (CA and CB respectively), total chlorophyll content (Ctotal) and also the ratio between chlorophyll a and b (CA_CB).

3. RESULTS

It is possible to observe in the PCA for the sorghum seedling (Fig 3.1) that the responses of the analyzed parameters were similar for the effect of NaCl doses and priming in the two cultivars. The cultivar DKB 540 when evaluated the PCA that compares the types of priming showed a greater grouping near axis 1 (26%). The Clustering of the variables analyzed indicates that there is an effect of the conditions (priming or NaCl dose) on the parameters analyzed. The PCA that analyzes the effect of NaCl dose shows that there is a cluster that is far from the others (240mM) being more limiting to the variables. The values of proline content (Fig. 3.2) show us that the increase of this osmolyte is directly related to the tolerance observed at the highest dose of NaCl. At the highest dose of salt there is a considerable increase in the concentration of this molecule closely

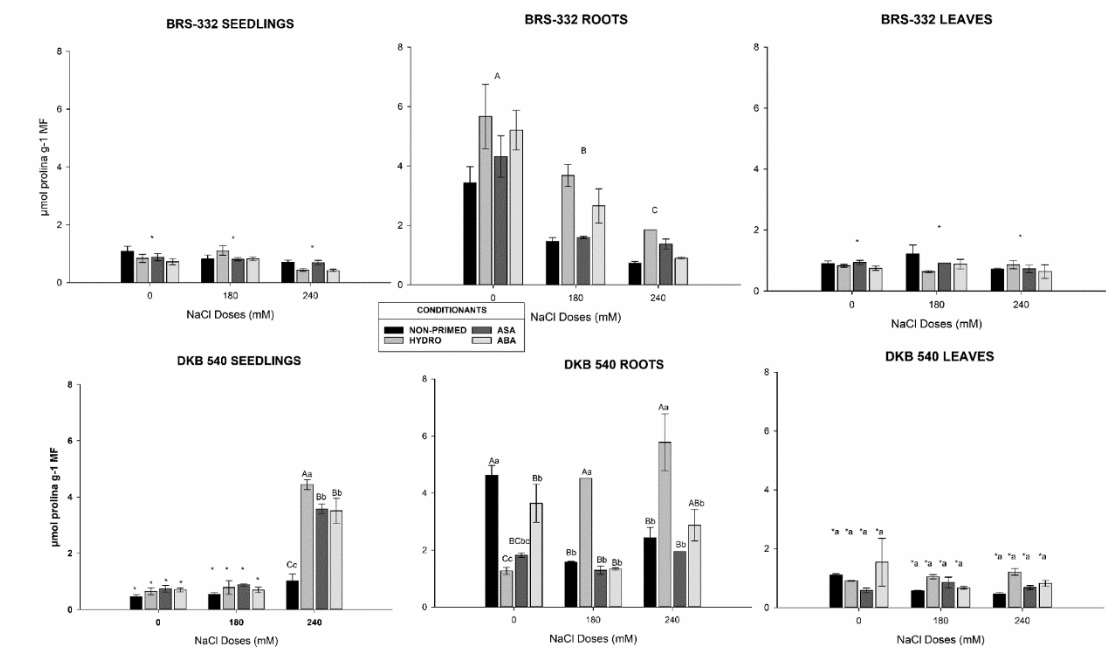
The cultivar BRS-332 showed a cluster closer to axis 1 (30.8%) for seeds that were unprimed or primed with ASA, indicating that the proximity of these two conditions had a greater effect on the variables analyzed. When analyzing the PCA for the NaCl doses it is possible to observe the formation of a cluster that is closer to the data of Pro, MDA and craiz indicating that the 240mM dose had a more detrimental effect on these variables while the control (0) and 180mM doses did not promote changes in the analyzed parameters. These results for proline (Fig. 3.2) as well as the results for MDA (suppl. Material 1) and hydrogen peroxide (suppl. Material 2) show that there is no increase that indicates oxidative damage. The same can be observed for Fv/Fm (Suppl. Material 5), where in both salt doses there are no changes, indicating little influence of the salt dose on this parameter.

Figure 3.1- Principal component analysis for evaluated parameters: Proline content (Pro), total amino acid content (AA) total soluble sugars (TSA) and reducing sugars (RA) content, primary quantum yield of photosystem II (Fv/Fm), electron transport quantum yield of QA- to intersystem electron acceptors (Phi_E0), primary photochemical maximum quantum yield at t = 0 (Phi_P0), reoxidation of QA- via electron transport in an active reaction center (ETo/RC) and electron absorption performance index (PI_abs) of sorghum seedlings



Source: Ferreira (2022)

Figure 3.2- Proline contend in seedling, roots and leaves of cultivars BRS-332 and DKB 540, primed with ascorbic acid (100µM), abscisic acid (100µM), hydro priming and non-primed subjected to salinity (0, 180, and 240 mM NaCl), Bars represent means ± standard error. Upper case letters compare the difference between the applied priming, and lower case letters compare the osmotic potentials or NaCl doses (n=4). Means followed by the same letters or Ns (non-significant) do not show significant difference by Tukey test at 5% significance level.



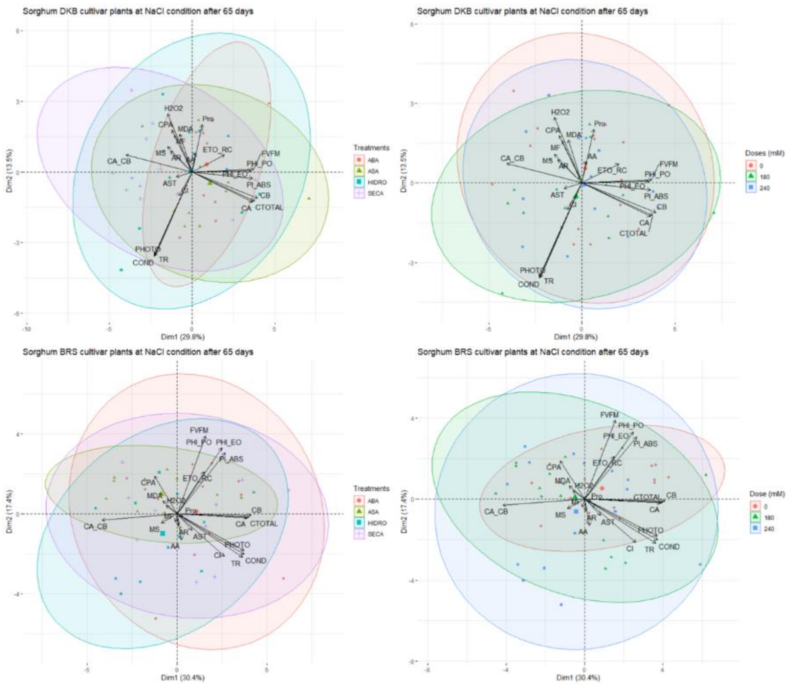
Source: Ferreira (2022)

Unlike the effects of priming and doses on the seedlings, it is possible to observe in plants at 65DAG that there are differences in relation to the parameters analyzed in the PCA (Fig. 3.3).

The drought tolerant cultivar DKB-540 showed a varied behavior according to the type of priming applied, and it is possible to observe that there is proximity of the data to axis 1 (29.8%) and the formation of clusters that overlap. The grouping that presents a smaller dispersion of the data is the one referring to ABA, the seed plants that were primed with this hormone presented a smaller variation in relation to the other types of priming or even seeds that did not undergo any treatment. Analyzing the effects of the NaCl doses it is possible to observe that there is the formation of heterogeneous groups, demonstrating that the effects on the cultivar were similar when compared to the control dose (0).

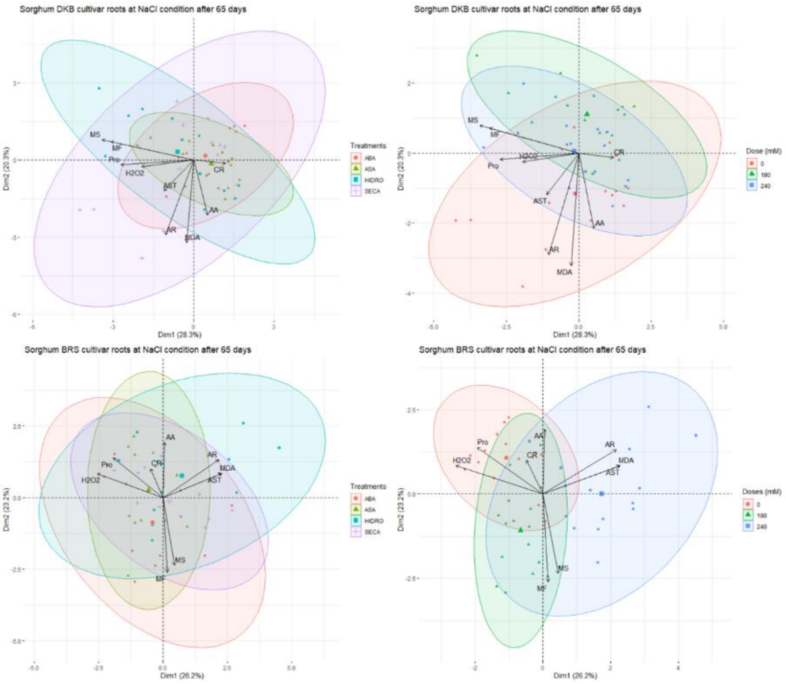
In the leaves of plants at 65 DAG it is possible to observe that there is no increase in proline (Fig. 3.2), however, there is accumulation of reducing sugars (suppl. Material 4) in plants, independent of the type of priming. It is also possible to observe a considerable increase in the content of H2O2 (suppl. Material 2) and MDA (suppl. Material 1). The effects on the parameter Fv/Fm, (suppl. Material 5) were not significant for DKB plants, even those that were not exposed to priming.

Figure 3.3- Principal component analysis for evaluated parameters: Proline content (Pro), total amino acid content (AA) total soluble sugars (TSA) and reducing sugars (RA) content, primary quantum yield of photosystem II (Fv/Fm), electron transport quantum yield of QA- to intersystem electron acceptors (Phi_E0), primary photochemical maximum quantum yield at t = 0 (Phi_P0), reoxidation of QA- via electron transport in an active reaction center (ETo/RC) and electron absorption performance index (PI_abs),photosynthesis (Photo), stomatal conductance (Cond), transpiration (TR) and internal carbon content (CI), chlorophyll a and b contents (CA and CB respectively), total chlorophyll content (Ctotal) and chlorophyll a and b ratio (CA_CB). of sorghum plants at 65 days of evaluation (pre anthesis phase).



Source: Ferreira (2022)

Figure 3.4- Principal component analysis for evaluated parameters: Proline content (Pro), total amino acid content (AA), total soluble sugars (TSA) and reducing sugars (RA), hydrogen peroxide (H2O2), root length (CR) MDA content (MDA), Fresh weight (MF) and Dry weight (MS) of sorghum plants roots.



Source: Ferreira (2024)

Figure 3.4 shows the principal components analysis for the effects of priming and NaCl dose for the roots of plants at 65 days after germination (65DAG). To perform this PCA the biochemical analysis data previously used were adopted, as well as the biometric data.

It is possible to observe for DKB 540 that when analyzing the parameters within the PCA for the type of priming that there is the formation of 4 clusters and the one that is closest to axis 1 (28.3% of the data) is the cluster represented by the priming with the antioxidant molecule ASA, followed by the priming with the phytohormone ABA. Similarly to the PCA where the effect of priming was evaluated, the PCA where the effects of the salt doses were evaluated allowed us to observe that there is a correlation between the biochemical and biometric parameters, especially for the control dose, which presented the greatest effects on the parameters evaluated.

The drought sensitive cultivar (BRS-332) shows the effects of priming with ascorbic acid, since it is possible to observe the grouping of the closest variables for this type of priming. It is possible to observe a greater dispersion of the data for hydropriming and the seeds that did not undergo priming. When analyzing the PCA for the NaCl doses, it is possible to observe a more dispersed grouping for the highest dose (240mM) while the control and 180mM doses allow observing a smaller effect on the analyzed variables.

4. DISCUSSION

According to the results obtained, it is possible to verify that (i) the effects of priming extend to the seedlings, however it is not possible to affirm that these effects are found in the plants; (ii) the effect of priming was able to approximate the tolerant and sensitive cultivars, independently of the type of priming and the dose of NaCl imposed on the plants. Based on our results, it is possible to infer that the priming effects found in seeds extend to the phenological stage of seedling, and although it is possible to observe advantages of the drought sensitive cultivar (BRS-332) when subjected to stressful salinity conditions.

The changes observed in the plants during the different phenological stages lead to believe that there is an evocation of the priming memory, that is, the effects found in the seedlings could be identified in the plants at 65DAG through the recovering metabolic changes imposed during the priming procedures. The biochemical alterations are mostly divergent from each other, the cultivar BRS-332 presents a higher reducing sugar metabolism, while the cultivar DKB 540 has a higher proline metabolism, these two attributes may be related to osmotic adjustment.

The observed effects of the priming lead us to some markers that present themselves as efficient in promoting tolerance to salt stress. The contents of proline and carbohydrates (total soluble sugars and reducing sugars) were those that could be pointed out as responsible for promoting the homeostasis of the plants.

It was possible to observe that there was an increase in the levels of hydrogen peroxide and MDA according to time (suppl material 1), with the highest levels of these ROS and this marker of oxidative stress being observed in the plants, for both cultivars. Since the effects of salt stress occur due to the dissociation of Na+ and Cl- ions, causing an imbalance in ROS formation and leading to oxidative stress, it culminates mainly in membrane damage, detectable through the increase in MDA levels. Under oxidative stress conditions, the accumulation of this marker is important to understand the extent of damage, however it was not possible to determine that the concentration found (supplementary material) indicate extensive oxidative damage capable of compromising the development of sorghum plants, regardless of the phenological stage adopted. The application of priming, independent of molecule or hydropriming, shows that the use of this technique is efficient in circumventing this situation.

The two cultivars (DKB 540 and BRS-332) had a similar responses analyzing these two parameters, both for seedlings and for roots and plants analyzed.

Some works (Iqbal et al., 2012 and Farooq et al., 2013) point out the efficiency of priming with ABA and ASA in promoting the mitigation of oxidative stress. Taking into account the H2O2 formation sites, the application of priming under salt stress conditions may have occurred mainly due to an osmotic adjustment and maintenance of stomatal opening. Considering the lower peroxide concentration in the seedlings of the two cultivars it is possible to attribute the control of the H_2O_2 concentration to the application of priming (Suppl. Material 2), the content observed in both plants under the higher NaCl conditions is similar between drought tolerant (DKB 540) and drought sensitive (BRS-332) cultivars.

Another relevant point to be highlighted is the accumulation of sugars (suppl. material 3 and 4) and amino acid proline (Fig. 2). These molecules are widely pointed out in promoting osmotic adjustment, guaranteeing that there are conditions in the maintenance of homeostasis. The application of priming with 100µM of ASA and ABA or even the application of hydropriming were efficient in guaranteeing an accumulation of these molecules. This calls attention to the fact that under the most stressful condition there is a greater accumulation of proline for the sensitive cultivar (BRS-332), which makes us believe that the efficiency of priming in promoting tolerance to salt stress was due to the greater accumulation of compatible osmolytes. Another relevant point to be pointed out is the accumulation of proline in roots of both cultivars, this structure being the first to have contact with the presence of NaCl in the soil and since this perception is closely linked to osmotic stress, the accumulation of this osmolyte may occur.

Another important parameter that serves as a strong indicator of tolerance is chlorophyll a fluorescence (Supll. Material 6 and 7). This technique allows us to understand the effects of salt stress on the photochemical step of photosynthesis. The results obtained through the evaluation of fluorescence allow us to infer that there are improvements in the photochemical stage due to the application of priming, since the results are close to the control condition. The Fv/Fm parameter presented alterations throughout the experiment, indicating that there were alterations but they could be circumvented. The application of priming allowed that, until 65 days of evaluation, there was a maintenance of Fv/Fm values (Suppl. material 5). However, there was a decrease in Fv/Fm for the cultivar BRS-332 in the control condition in the first evaluation, indicating the effects of priming because there was a recovery, indicating that it was possible to recovery the photochemical stage due to the application of priming with the phytohormone ABA. At the end of the experiment, the maintenance of the values close to 0.8, indicating that there are conditions for the sorghum plants to get around the salt stress in the 240mM dose.

The parameters that reflect the photosynthetic metabolism of the plants were evaluated at the end of the experiment (Suppl material 8 and 9). It is possible to observe that there are higher values for the variables Photo, stomatal conductance (gs) transpiration (tr) and internal carbon (IC) for the cultivar BRS-332 when the plants are submitted to the control dose and 240mM of NaCl, being followed by the dose of 180mM when submitted to priming with ABA, ASA and hydropriming. These results are similar to those of the DKB 540 cultivar, indicating that it was possible to obtain effects in maintaining plant homeostasis, possibly due to the accumulation of proline and sugars.

In general, it is possible to indicate a metabolic and redox adjustment of the plants, and the priming with the antioxidant molecule AsA and ABA as well as hydropriming were efficient in guaranteeing these effects.

5. CONCLUSIONS

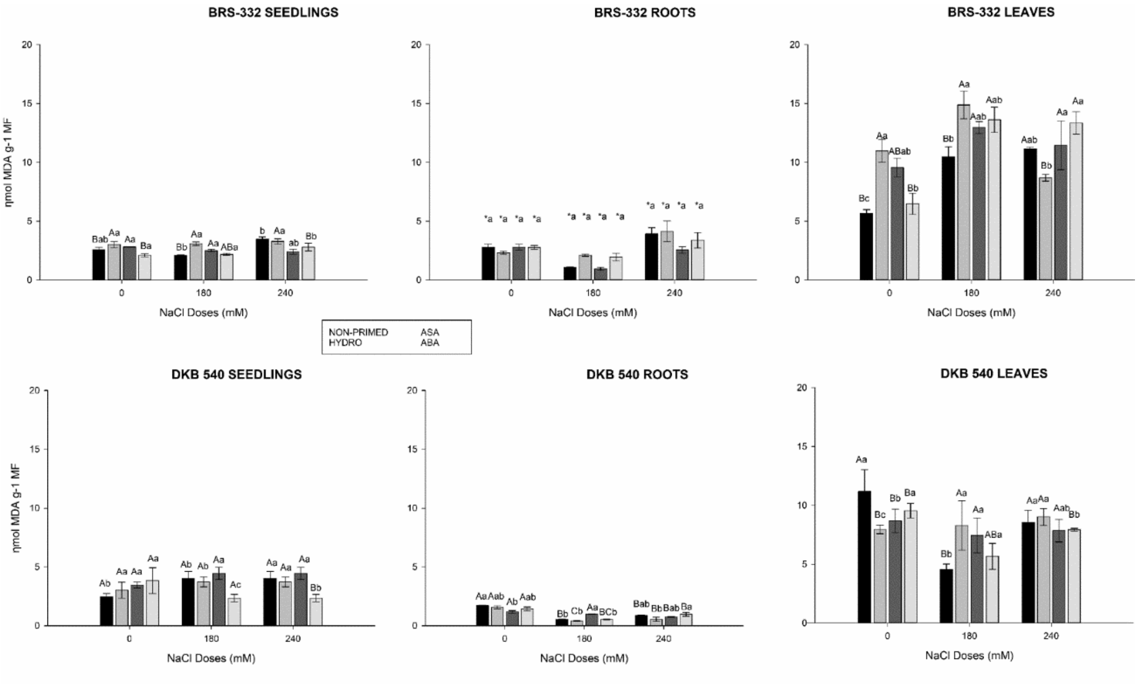
Based on our results, it is possible to infer that there are benefits from the application of priming in seeds that will reflect favorable results in plantlets and plants. It is important to emphasize that the application of priming aims to provide conditions for the germination and development of plants under unfavorable conditions through the recruitment of resources prior to the imposition of the stressful condition. In our experiment, there were some improvements with priming of the variables analyzed for the cultivar BRS-332 under stress conditions. This gives us clues that the application of priming was effective in promoting better conditions for the development of this cultivar sensitive to drought. Therefore, the priming, either with AsA, ABA or even hydropriming is an effective tool in promoting better conditions for the growth and development of sensitive crops under unfavorable conditions. Moreover, it is important to note that several markers can be adopted as indicators of the effects of priming in different phenological states, and it is important to understand the effects of memory promoted by this application through evaluations of epigenetics, thus ensuring that the metabolic improvements obtained serve as guidelines for the genetic improvement of agricultural species of interest.

6. AKNOWLEDGMENTS

To Professor Eduardo Gusmão Pereira for the equipment’s used in the evaluation of Gas exchange and chlorophyll fluorescence parameters. To Moreira, M.B. and Pereira, A.A.S for the support during the experiment period.

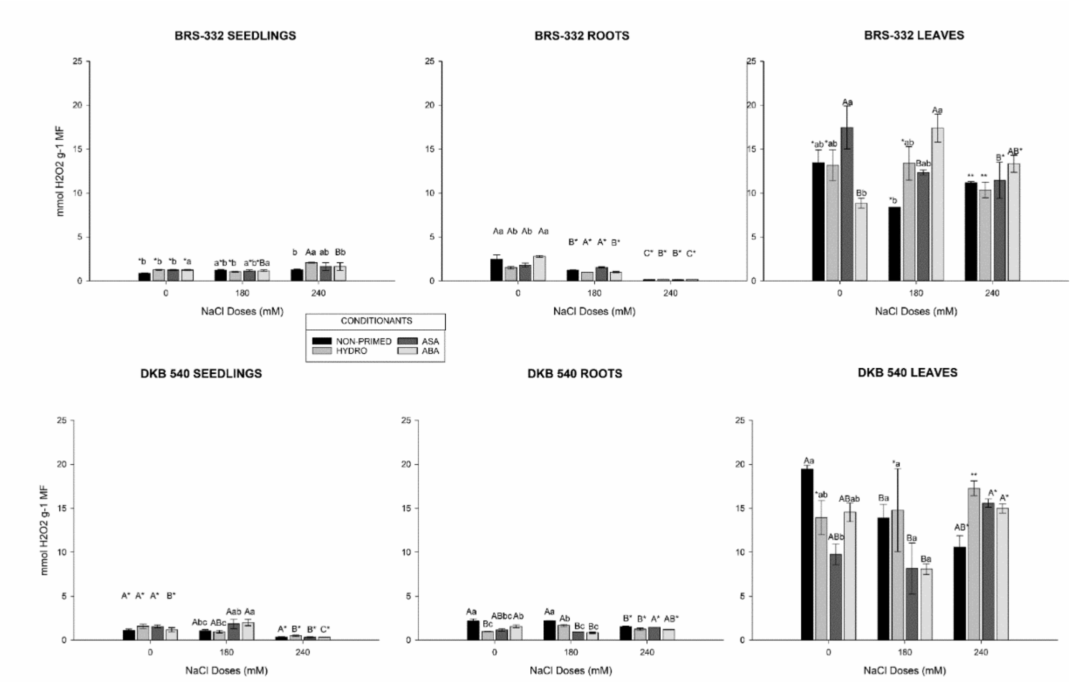
7. SUPPLEMENTARY MATERIAL

Suppl. Material 1: MDA contend of cultivars BRS-332 and DKB 540, primed with ascorbic acid (100µM), abscisic acid (100µM), hydro priming and non-primed subjected to salinity (0, 180, and 240 mM NaCl), Bars represent means ± standard error. Upper case letters compare the difference between the applied priming, and lower case letters compare the osmotic potentials or NaCl doses (n=4). Means followed by the same letters or Ns (non-significant) do not show significant difference by Tukey test at 5% significance level.



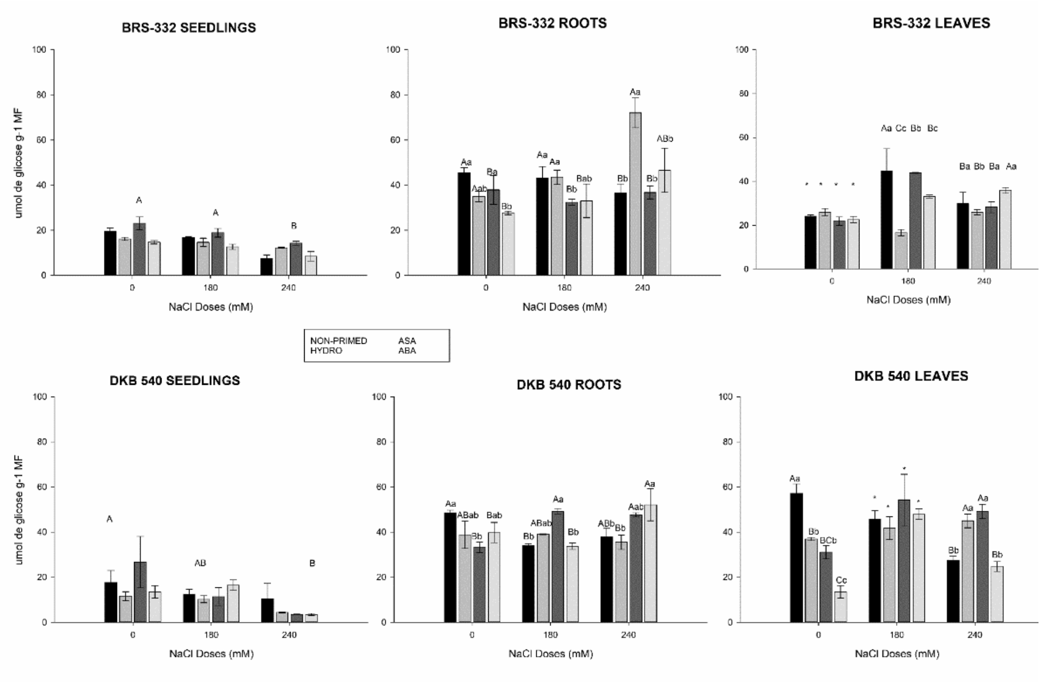
Source: Ferreira (2022)

Suppl. Material 2: H_2O_2 contend of cultivars BRS-332 and DKB 540, primed with ascorbic acid (100µM), abscisic acid (100µM), hydro priming and non-primed subjected to salinity (0, 180, and 240 mM NaCl), Bars represent means ± standard error. Upper case letters compare the difference between the applied priming, and lower case letters compare the osmotic potentials or NaCl doses (*n*=4). Means followed by the same letters or Ns (non-significant) do not show significant difference by Tukey test at 5% significance level.



Source: Ferreira (2022)

Suppl. Material 3: Total soluble sugars contend of cultivars BRS-332 and DKB 540, primed with ascorbic acid (100µM), abscisic acid (100µM), hydro priming and non-primed subjected to salinity (0, 180, and 240 mM NaCl), Bars represent means ± standard error. Upper case letters compare the difference between the applied priming, and lower case letters compare the osmotic potentials or NaCl doses (*n*=4). Means followed by the same letters or Ns (non-significant) do not show significant difference by Tukey test at 5% significance level.



BRS-332 SEEDLINGS

BRS-332 ROOTS

BRS-332 LEAVES

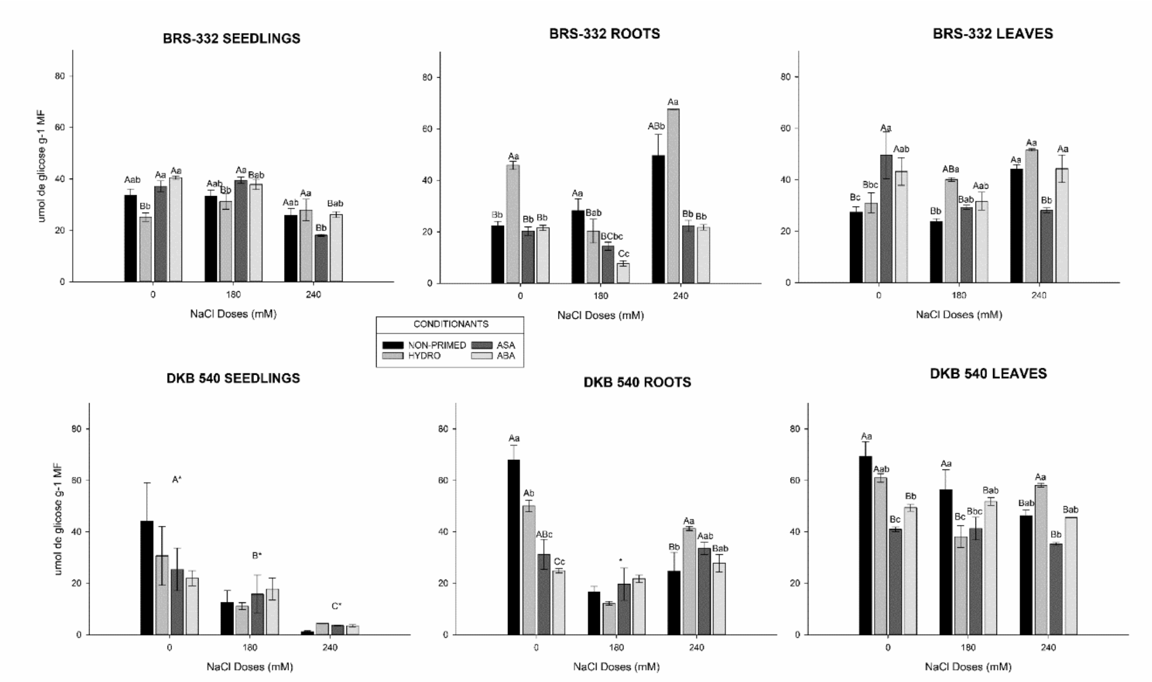
DKB 540 SEEDLINGS

DKB 540 ROOTS

DKB 540 LEAVES

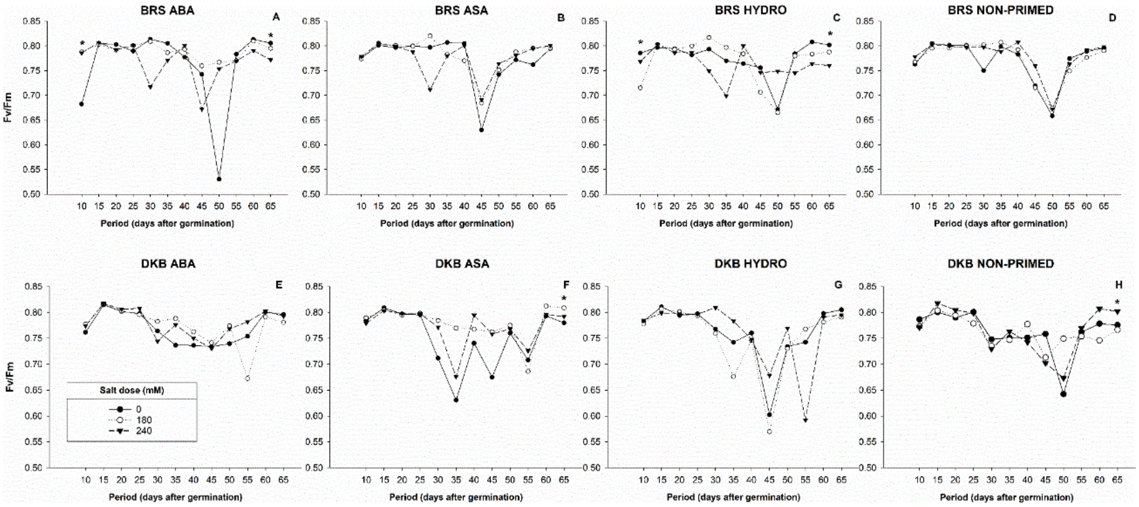
Source: Ferreira (2022)

Suppl Material 4: Reducing sugars contend of cultivars BRS-332 and DKB 540, primed with ascorbic acid (100µM), abscisic acid (100µM), hydro priming and non-primed subjected to salinity (0, 180, and 240 mM NaCl), Bars represent means ± standard error. Upper case letters compare the difference between the applied priming, and lower case letters compare the osmotic potentials or NaCl doses (*n*=4). Means followed by the same letters or Ns (non-significant) do not show significant difference by Tukey test at 5% significance level.



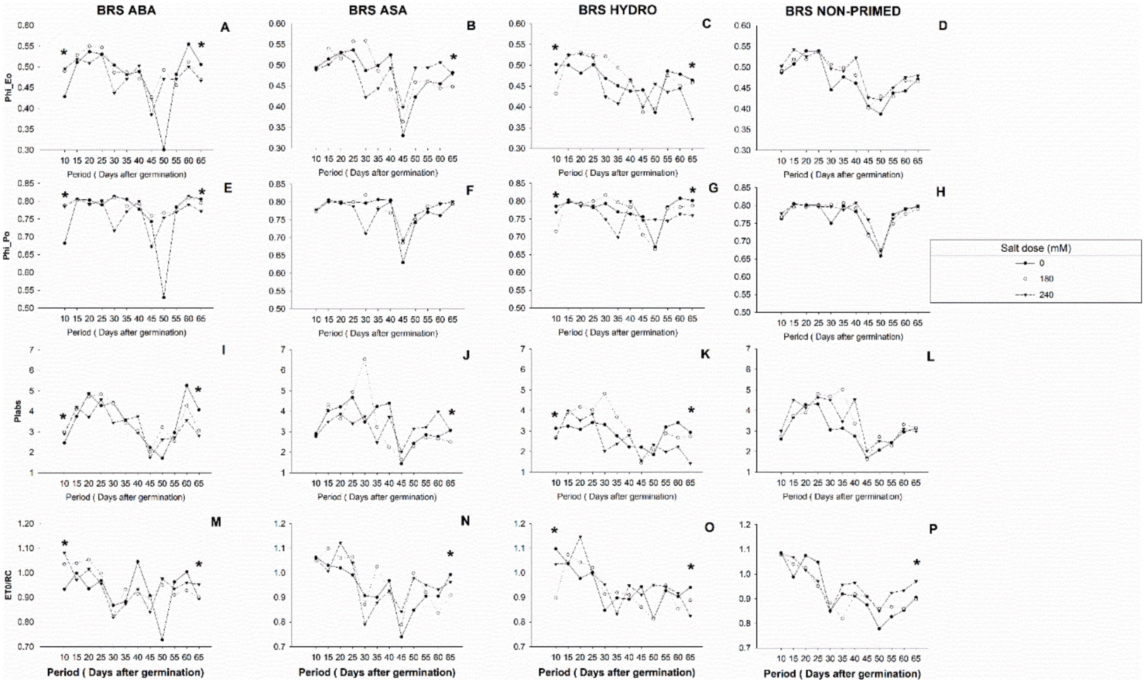
Source: Ferreira (2022)

Suppl. Material 5: Maximum quantum yield of PSII (*Fv/Fm*) Analyzes performed on leaves of cultivars BRS-332 and DKB 540, primed with ascorbic acid (100µM), abscisic acid (100µM), hydro priming and non-primed, subjected to salinity (0, 180, and 240 mM NaCl)(*n=4*). Asterisks represents the statistical differences at P<0.05 (Tukey test).



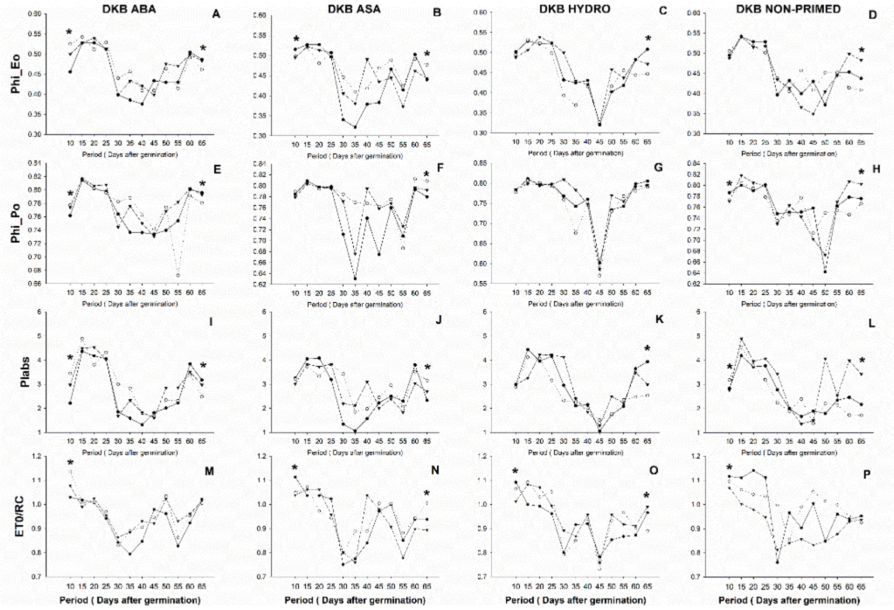
Source: Ferreira (2022)

Suppl. Material 6: Transient chlorophyll *a* parameters, electron transport quantum yield of QA- to intersystem electron acceptors (Phi_E0), primary photochemical maximum quantum yield at t = 0 (Phi_P0), electron absorption performance index (PI_abs) and reoxidation of QA- via electron transport in an active reaction center (ETo/RC). Analyzes performed on leaves of cultivar BRS-332, primed with ascorbic acid (100µM), abscisic acid (100µM), hydro priming and non-primed subjected to salinity (0, 180, and 240 mM NaCl)(*n=4*). Asterisks represents the statistical differences at P<0.05 (Tukey test).



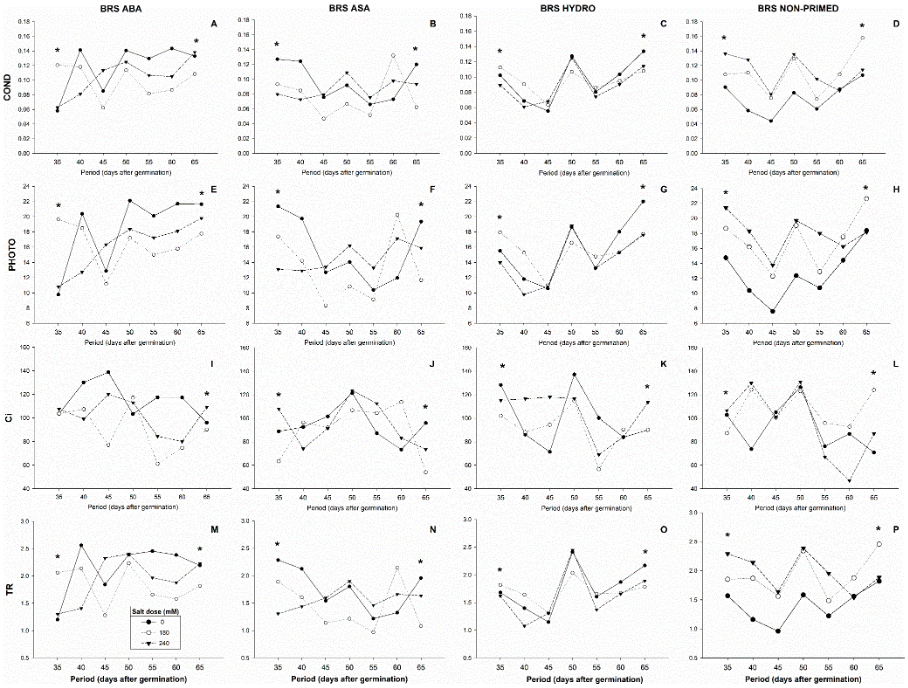
Source: Ferreira (2022)

Suppl. Material 7: Transient chlorophyll *a* parameters, electron transport quantum yield of QA- to intersystem electron acceptors (Phi_E0), primary photochemical maximum quantum yield at t = 0 (Phi_P0), electron absorption performance index (PI_abs) and reoxidation of QA- via electron transport in an active reaction center (ETo/RC). Analyzes performed on leaves of cultivar DKB 540, primed with ascorbic acid (100µM), abscisic acid (100µM), hydro priming and non-primed subjected to salinity (0, 180, and 240 mM NaCl)(*n=4)*. Asterisks represents the statistical differences at P<0.05 (Tukey test).



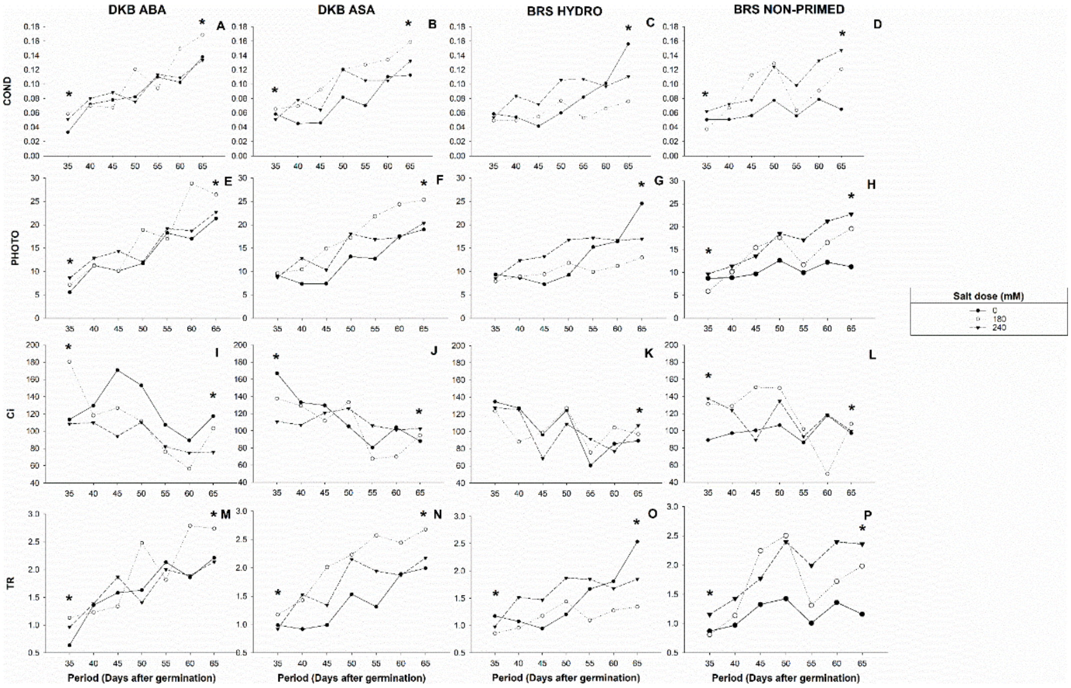
Source: Ferreira (2022)

Suppl. Material 8: Stomatal conductance (Cond), Photosynthesis (Photo), internal carbon concentration (Ci) and transpiration (Tr) Analyzes performed on leaves of cultivar BRS-332, primed with ascorbic acid (100µM), abscisic acid (100µM), hydro priming and non-primed, subjected to salinity (0, 180, and 240 mM NaCl) (*n=4)*. Asterisks represents the statistical differences at P<0.05 (Tukey test).



Source: Ferreira (2022)

Suppl. Material 9: stomatal conductance (Cond), Photosynthesis (Photo), internal carbon concentration (Ci) and transpiration (Tr) Analyzes performed on leaves of cultivar DKB 540, primed with ascorbic acid (100µM), abscisic acid (100µM), hydro priming and non-primed, subjected to salinity (0, 180, and 240 mM NaCl)(*n=4*). Asterisks represents the statistical differences at P<0.05 (Tukey test).



Source**:** Ferreira (2022)

8. ACKNOWLEDGEMENTS

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1. CONCLUDING REMARKS

The effects of salinity are a factor that imposes serious restrictions on the germination and growth of different crops. Among the scientific advances in the field of seed science, the application of priming appears as a prominent tool.

Seed priming provides a range of metabolic changes that allow and understanding how the application of priming in seeds can overcome this condition. understanding which and how these changes occur is an important step is an important step for breeding programs, aiming mainly at the access to data from the point of view of plant physiology, in order to promote food security, through more responsive plants in areas where salinity can be a limiting factor for obtaining optimal results in sorghum productivity.

In this work, it was possible to identify biochemical markers highly responsive to salt stress. The identification of these markers are very important because it gives indications that the application of priming with different molecules is an effective practice to be adopted and, above all, an accessible practice that allows overcoming this stress during the plant reproductive processes.

One of the highlights of this work was the identification of how the role of compatible osmolytes, in our case proline, and carbohydrates, is are a key factor to be explored. In biochemical analyses performed on seeds, as well as on plants, these molecules were able to promote benefits, bringing germination and establishment of plants from of a cultivar referenced as susceptible to drought (BRS-332), that accumulated a high content of sugars (TSS and RS), close to a tolerant cultivar, in this case the hybrid DKB 540.

We could observe that the priming with ascorbic acid and ABA were able to provide these improvements, but an important highlight is the adoption of hydropriming. The application of this type of priming is an indication that even the use of a simple resource can be an advance in obtaining better results in seed germination.

Besides the benefits found in seeds, it is important to point out that those found in seedlings and plants are able to present us with a resource called priming memory. The benefits obtained in the synchronization and increase of germination under osmotic and salinity stress conditions, as the accumulation of proline and carbohydrates, could be recruited by the plants, promoting improvements in physiological aspects, such as better results in the photosynthetic metabolism, as well as improvements in the photochemical mechanism and in gas exchange, these benefits could be promoted through a better osmotic adjustment or even by the role of proline in overcome the effects of the ROS formation.

Thus, it is worth noting that the application of priming can promote cross-tolerance, one of the objectives when applying this technique, since, under unfavorable conditions where one or more stress factors may be occurring, the different types of priming could guarantee that the establishment of the culture was not affected by the stresses occurring.

Thus, at the end of this work, it is possible to deliver results that allow the advancement of knowledge of an accessible and low-cost practice, but which allows satisfactory results to be obtained. The results presented here can serve as a reference in the advancement of the study of the priming technique, by presenting answers on how seeds respond to high salinity, since it was possible to understand the main effects of this condition, as well as answer a gap in scientific knowledge, since it elucidates how plants and plants can benefit from the application of this technique.